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SPECIAL LISTING

CURRENT CANCER RESEARCH

on

NITROSO COMPOUNDS

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service ● National Institutes of Health ●
National Cancer Institute

A SERVICE OF THE
INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB)

Dear Cancer Researcher:

This **SPECIAL LISTING** of Current Cancer Research Projects is a publication of the INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB) Program of the National Cancer Institute. Each **LISTING** contains descriptions of ongoing projects in one selected cancer research area. Approximately 50 different cancer research areas are covered by a separate **SPECIAL LISTING**.

The present series of **SPECIAL LISTINGS** is a third edition, and begins with number 201. This series will update the second edition which included **SPECIAL LISTINGS** numbered 101 through 147. Depending on the total number and types of cancer research summaries received, the third edition will have at least 47 titles, and possibly a few more. In most cases, each third edition **SPECIAL LISTING** will replace the exact subject area of a second edition **LISTING** (SL 201 replacing SL 101, etc.). Whenever the number and variety of summaries requires a change in this procedure, a notation will be made in the specific **LISTINGS** affected.

The purpose of these **LISTINGS** is to promote and facilitate the exchange of information among cancer scientists. The project descriptions in the **LISTINGS** are provided by cancer scientists from more than 50 different countries. Researchers are urged to prepare accurate summaries which will be rapidly processed for inclusion in the CANCERPROJ data base as well as future **SPECIAL LISTINGS**.

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CURRENT CANCER RESEARCH

I. EFFECT OF NITROSO COMPOUNDS ON NUCLEIC ACIDS

A. EFFECT OF NITROSAMINES ON NUCLEIC ACIDS

1. MECHANISM OF NITROSAMINE ALKYLATION OF DNA AND RNA

Archer, M. C., Ontario Cancer Inst., *500 Sherbourne St., Toronto, Ontario*, M4X 1K9, Canada

The overall objective of the proposed research is to define the mechanism of alkylation of cellular targets such as nucleophilic sites in DNA and RNA, by carcinogenic nitrosamines. In addition to direct transfer of intact alkyl groups to cellular nucleic acids, methylation appears to be a reaction common to several dialkylnitrosamines and possibly cyclic nitrosamines. Using N-nitrosodipropylamine and N-nitrosopiperidine as examples of the two classes of nitrosamines, the mechanism of formation of methylating agents by these compounds and their beta-oxidized derivatives in isolated rat liver preparations will be investigated.

2. STUDY OF THE ROLE OF THE REACTIONS BETWEEN NITROSAMINES AND DNA IN THE INDUCTION OF CANCER

Swann, P. F., Kyrtopoulos, S. A., Courtauld Inst. of Biochemistry, *London, England, United Kingdom*

OBJECTIVE: To test the hypothesis that the cancers produced by nitroso compounds are a consequence of the reaction between the active metabolites of nitroso compounds and the O6 position of guanine in DNA.

APPROACH: The hypothesis is being tested by studies of the correlation between the extent of formation and enzymic repair of O6 alkylguanine in various organs of the rat after treatment with the nitrosamines, and the carcinogenic potency of single, divided and repeated doses of these nitrosamines toward each organ.

3. PROTEIN AND NUCLEIC ACID METABOLISM IN GROWTH REGULATION, DIFFERENTIATION, AND TUMOR INDUCTION AND EFFECTS OF CARCINOGENS

Hultin, T., Wenner Gren Inst. for Experimental Biology, Dept. of Cell Physiology, *Norrtullsgatan 16, S11345 Stockholm, Sweden*

OBJECTIVE: To elucidate the rapid inhibition of protein synthesis and accumulation of monoribosomes in the liver following dimethylnitrosamine (DMNA) administration.

APPROACH: The inhibition of protein synthesis by DMNA is accompanied by: (a) a rapid decrease in postmicrosomal poly(A) positive RNA, probably representing free mRNP particles, and (b) a decreased activity of subcellular ('S-30') liver systems in forming 80S initiation complexes in vitro. The molecular mechanism of these effects of the carcinogen will be investigated along the following lines: (1) Is the primary effect of DMNA on liver protein synthesis related to disturbed mRNP/ribosome interaction in the initiation of new protein chains, or to a selective degradation of non-polyribosomal mRNA? In the former case reinitiation in vitro by use of polyribosomal mRNA would be inhibited to the same extent as primary initiation. (2) Is the DMNA effect associated with alterations at the level of mRNP particles? Is there evidence of membrane-associated mRNP in liver cells, which might be preferentially exposed to DMNA metabolities? (3) Can isolated hepatocytes be used to elucidate the early effects of DMNA on mRNA translation? (d) Biochemical characterization of ribosomal initiation complexes in liver systems.

4. PATHOLOGY OF CHEMICAL CARCINOGENESIS

Smuckler, E. A., Clawson, G. A., James, J. L., University of California, San Francisco Campus, School of Medicine, Dept. of Pathology, *551 Parnassus Ave., San Francisco, California*, 94122, United States of America

The mechanisms underlying chemically mediated malignant transformation are to be explored. Model systems to be used include in vivo exposure of rodents to thioacetamide, ethionine, alkylnitrosamines, and amino azo dyes. In vitro exposure of hepatocyte cultures to these same agents will also be employed. Previous data show a modification of nuclear restriction of RNA species in liver cells exposed to carcinogens, potentially representing an initiating or a promoting event, or both. The major areas to be investigated include: (1) An analysis of the cell-free model of nuclear RNA transport to determine the role of high-energy bond hydrolysis as a driving force, and to assay for the significance of selection of RNA species both for secretion and for back-diffusion to the nucleus; (2) an analysis of in vivo restriction in the two-stage model of hepatocarcinogenesis to learn whether 'nuclear leakage' is related to initiation or to promotion; and (3) a continuing effort to purify DNA-dependent RNA polymerase from rat liver, especially by separating the nicking enzyme from the polymerase in order to clearly assay for initiation. These experiments will be used to test the hypothesis that malignant transformation involves alterations in the transcription and processing of RNA, at least as a basis for the phenotypic expression in cancer.

5. METABOLISM OF DIALKYL AND CYCLIC NITROSAMINES AND GENETICS OF RESPONSE TO ENZYME INDUCERS - HYDROCARBON-NITROSAMINE SYNCARCINOGENESIS

Arcos, J. C., Argus, M. F., Tulane University of Louisiana, School of Medicine, Dept. of Medicine, *1430 Tulane Ave., New Orleans, Louisiana*, 70112, United States of America

Previous studies with 14C-labeled dimethylnitrosamine (DMN), diethylnitrosamine and N-nitrosopiperidine suggest that the molecular size of the higher nitrosamines may be a stereochemical limiting factor in microsome-catalyzed in vitro binding to whole DNA. Hence, we will investigate in a standardized assay system the binding of nonradioactive forms of these nitrosamines to 14C-labeled purine and pyrimidine nucleosides (using microsomes from normal and inducer-pretreated animals), in which reaction the role of steric hindrance would be minimized. In view of the unexpected, diametrically opposite effects of pregnenolone-16alpha-carbonitrile and beta-naphthoflavone - two strong repressors of DMN-demethylase I activity - on DMN-induced carcinogenesis, we will determine the effect of pretreatment with these two agents on: (a) The binding of DMN to DNA in vitro and in vivo; (b) the mutagenicity of DMN in a host-mediated assay system; (c) the LD50 of DMN; and (d) DMN-demethylase II activity. In other experiments the possible role of microsomal amine-oxidase (in whole microsomes and in purified form) in the dealkylation of DMN and other nitrosamines will be explored.

6. PERSISTENCE OF ALKYLATED DNA IN CARCINOGENESIS

Pegg, A. E., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Physiology, *500 University Dr., Hershey, Pennsylvania*, 17033, United States of America

The aim of the research is to discover the underlying reactions responsible for the carcinogenicity of nitroso com-

pounds and to ascertain whether there are protective mechanisms by which the cell can minimize the damage produced by these agents. The hypothesis that carcinogenesis is due to the formation and persistence in DNA of the promutagenic base, O6-methylguanine, is being studied. The reactions responsible for the formation of, and subsequent loss of this product are being studied in a number of different tissues known to differ in their susceptibility to the carcinogenic insult produced by the nitrosamines. At present, all of the data collected is consistent with the hypothesis and it appears that the relative resistance of the liver to carcinogenesis is due to the high efficiency with which this abnormal purine can be removed from liver DNA.

The studies are being carried out using dimethylnitrosamine which produces liver and kidney tumors in rats, N-methyl-N-nitrosourea which produces tumors in the nervous system and in other organs and 1,2-dimethylhydrazine which gives rise to tumors in the colon.

The enzyme responsible for the removal of O6-methylguanine from rat liver has been partially purified and the properties of this enzyme are currently under investigation.

BIBLIOGRAPHIC REFERENCES: Pegg, A.E. Enzymatic removal of O6-methylguanine from DNA by mammalian cell extracts. Biochem. Biophys. Res. Commun. 84, 166-173, 1978. Stumpf, R., Margison, G.P., Montesano, R. and Pegg, A.E. Formation and loss of alkylated purines from DNA of hamster tissues after administration of dimethylnitrosamine. Cancer Res. 39, 50-54, 1979.

7. ANALYSIS OF CELLULAR EVENTS IN CHEMICAL CARCINOGENESIS

Becker, F. F., Lapeyre, J., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., *c/o Texas Medical Ctr., Houston, Texas,* 77030, United States of America

A major objective of this grant is the continued development of various chemical carcinogenic regimens which would make possible a more discrete and specific analysis of macromolecular interactions and cellular events in the evolution to malignancy. We will continue to examine the relative sensitivity of the various phases of the cell cycle in the action of hepatocarcinogens. In particular, we will focus upon the effect of accumulating metaphase figures by the use of colchicine as a means of increasing the sensitivity of the mitotic (M)-phase to the effects of DMN. Analysis of these results may make it possible to identify those components of a cell cycle which enhance sensitivity to carcinogens. In particular, since the chromatin of the M-cell is quite condensed, a heightened sensitivity would suggest the intervention of other cellular mechanisms such as transport into the cell itself, activation of the carcinogen or binding and damage of DNA. These factors will be examined by the use of labeled DMN, in uptake and binding studies and the alkaline-elution technique for determining DNA damage.

A continued examination of the synergistic effect of DMN on cells which have been exposed to AAF will also be accomplished by these techniques using labeled DMN.

A major effort will be aimed at developing the concept of the demand-hypothesis as a factor of the promotion of parenchymal carcinogenesis. In addition to analysis of the effects of proteinuria upon hepatocarcinogenesis, we will instigate a study of other intensive demands upon the liver such as increasing glucose utilization, protein turnover or challenging the liver with other agents which require detoxification.

BIBLIOGRAPHIC REFERENCES: Serum alpha-Fetoprotein in a Mouse Strain (C3H-Avy)with Spontaneous Hepatocellular Carcinomas. F.F. Becker, David Stillman, and Stewart Sell. Cancer Res.37:870-872, March 1977.

B. EFFECT OF NITROSOUREAS ON NUCLEIC ACIDS

8. ALKYLATION OF POLYNUCLEOTIDES IN VITRO AND IN VIVO

Fraenkel Conrat, B. A., Fraenkel Conrat, H. L., Spengler, S. J., University of California, Berkeley Campus, School of Letters & Science, Dept. of Molecular Biology, *Berkeley, California,* 94720, United States of America

This project is directed toward identifying the actual products of chemical reactions between alkylating agents which are mutagens and/or carcinogens and informational macromolecules, and then assessing the role of various modifications in producing biological effects. Specifically, we plan to study the products formed and their fate during replication, when the powerful carcinogen, ethylnitrosourea, acts on DNA in proliferating normal and xeroderma pigmentosum fibroblasts and on fetal rat brain and rat liver cells. We know from our recent work that N-nitroso ethylating agents primarily alkylate oxygens (in vitro and in vivo), including the phosphodiesters and base oxygens of C, U, T and G (and in the case of RNA, the 2'-O of ribose) and several of these are mutagenic events. The effects of oxygen substitution on the physical and biological properties of nucleic acids will be studied in several ways. After determining the stability of O-alkyl derivatives under a variety of conditions, we have developed appropriate methods for quantitating all the O-alkyl bases, 7-EtG, 3-EtG and ethyl-phosphotriesters in enzyme digests of 14C-ethyl-nitrosourea-treated DNA with a total of 2-6 ethyl groups per 10 to the 5th power DNA-P. At this level of alkylation, the cells show little cytotoxicity and can be replicated so that excision of each alkyl derivative can also be quantitatively determined. Our objective is to find whether there is a correlation between rates of excision or persistence of O-alkyl products and the biological effects observed with cells lacking UV repair enzymes (xeroderma pigmentosum fibroblasts) or susceptible to neoplastic transformation (fetal rat brain cells). We will also attempt to prepare homopolymers of O-alkyl pyrimidines in order to study their secondary structure, transcription and translation. The effect of O-alkyl substitution in translation will also be studied by examining the ability of modified codons to function in the binding of tRNA in the presence of ribosomes, or the ability of modified mRNA to be translated accurately in a wheat embryo cell-free system.

9. CELLULAR REPAIR OF POTENTIALLY MUTAGENIC DAMAGE

Ward, J. F., Lewis, H. L., University of California, Los Angeles Campus, Lab. of Nuclear Medicine & Radiation Biology, *900 Veteran Ave., Los Angeles, California,* 90024, United States of America

The objectives of this research are: (1) to develop highly sensitive serologic assays for DNA base damage, (2) to apply the assays to the study of cellular damage and its repair, and (3) to study the relation of base damage to carcinogenesis and mutagenesis. The serologic approach is important since current physical-chemical methods for the study of base damage at low doses and within the DNA macromolecule are inadequate. Serologic assays are highly sensitive and specific and permit quantitation of particular ionizing radiation damaged bases. Assay systems are being developed for quantitation of products in isolated DNA and for localization within cell preparations.

Antibodies have been prepared to the ionizing radiation products, 5-hydroxymethyluridine and 8,5'-cycloadenosine, and by phage neutralization assay yields have been measured in irradiated solutions of parent compounds under several conditions. Both products have been detected in irradiated DNA. We have developed a radioimmunoassay for UV photoproducts and production and repair of damage in HeLa cells has been followed. Antibodies have been prepared to O6-ethylguanosine and a highly sensitive method for the detection of this product in ethylnitrosourea treated DNA has been developed.

Repair studies will be extended to include ionizing radiation and chemical carcinogen damaged bases. Damage accumulation and repair will be correlated with survival and mutagenesis in cell systems.

10. ALKYLATING AGENTS, NITROSOUREAS - MOLECULAR PHARMACOLOGY

Ludlum, D. B., Archer, S., Tong, W. P., Abbott, P. J., Gombar, C., Union University, Albany Medical College, School of Medicine, Dept. of Pharmacology & Experimental Therapeutics, *47 New Scotland Ave., Albany, New York,* 12208, United States of America

Classical alkylating agents as well as the nitrosoureas evidently produce their therapeutic, as well as their mutagenic and carcinogenic effects by attacking the DNA of target cells. Therapeutic properties are probably related to the agent's ability to produce crosslinks in the DNA, and resistence may develop in cells that can repair this damage. The chemical nature of

such crosslinks will be identified in this project, and means will be sought to produce structural changes which are resistant to repair.

Synthetic polynucleosides can be studied separately. Since these polymers are effectively replicated by RNA polymerase, modified and unmodified polymers can be compared to determine which reactions are biologically significant. By obtaining data on different classes of agents - for example, classical alkylating agents in comparison with nitrosoureas - we will look for explanations of the clinically observed differences among these agents.

Using information obtained on the kinds of modifications produced by different classes of agents, new compounds which combine some of the features of each class will be synthesized and tested for their antitumor activity.

BIBLIOGRAPHIC REFERENCES: A Simplified Assay for Methotrexate in Biological Fluids Using a Crude Yeast Lysate. E.E. Weir, H. Kaizer, J. Rosenberg and D.B. Ludlum, Clin. Chim. Acta, 88, 207-213 (1978). Analysis of Methotrexate and 7 Hydroxymethotrexate by High Pressure Liquid Chromatography. J.L. Wisnicki, W.P. Tong and D.B. Ludlum, Cancer Treatment Reports, 62, 529-532 (1978).

11. DNA REPAIR IN CARCINOGENESIS

Cox, R., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *1030 Jefferson Ave., Memphis, Tennessee*, 38104, United States of America

The proposed research has been concerned with the interaction of chemical carcinogens with DNA and the consequences (damage and repair) of this reaction as it relates to the integrity of the cell. We have used mainly alkylating carcinogens that induce cancer in various tissues of the rat, depending upon the route of administration and dose. Data from the study of methylnitrosourea induced rat bladder and mammary gland cancer support the hypothesis that O6-methylguanine plays a vital role in the initiation of tumors in these organs. Methylnitrosourea was also demonstrated in the rat brain to bind specifically to DNA that was digested by DNAse I in whole nuclei. In this same system O6-methylguanine was shown to be more stable in the resistant regions of chromatin. These studies are continuing with a liver system where a single dose of the carcinogen is sufficient for the induction of cancer.

C. EFFECT OF MULTIPLE TYPES OF NITROSO COMPOUNDS ON NUCLEIC ACIDS

12. CHEMICAL CARCINOGENESIS (WITH EMPHASIS ON N-NITROSO COMPOUNDS)

Craig, A. W., O'Connor, P. J., Margison, G. P., Saffhill, R., Paterson Lab., Carcinogenesis Section, *550 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom*

OBJECTIVE: To establish the mode of action of carcinogenic N-nitroso compounds.

APPROACH: On the basis of a detailed knowledge of DNA reaction sites, following treatment with N-nitroso compounds in vivo, the relative stability and miscoding potential of particular lesions are being studied. The emphasis has been placed on extending our knowledge of the DNA repair system for O6-alkylguanines in which both acute and chronic administration of different N-nitroso compounds have been examined in rats and Syrian and Chinese hamsters. The effect of pretreatment with hepatocarcinogens, structurally unrelated to N-nitroso compound, is also being examined. Using an in vitro system it has been shown that the presence of O4-methylthymine in synthetic polynucleotides will cause miscoding with a DNA polymerase. Although this is a minor product, in vivo studies are being made to assess its significance in the carcinogenic process.

13. IMMUNOCHEMICAL APPROACH TO THE STUDY OF DNA-CARCINOGEN INTERACTION

Swann, P. F., Kyrtopoulos, S. A., Courtauld Inst. of Biochemistry, Dept. of Biochemistry, *London, England, United Kingdom*

OBJECTIVE: To develop specific immunochemical methods for the measurement of the concentration of carcinogen-DNA adducts in samples of DNA taken from the organs of animals treated with nitroso compounds. Use of immunochemical probes to study the distribution of carcinogen-DNA adducts in the different cells of an organ, and in different parts of the chromosome.

APPROACH: Antibodies against O6-methyl-2-deoxyguanosine-BSA conjugate have been raised in rabbits. These are being used in normal radioimmunoassay and immunoperoxidase techniques (preliminary report of a radioimmunoassay for O6-methyl-2-deoxyguanosine, Brit. J. Cancer, in press).

14. CORRELATIONS BETWEEN CELL PROLIFERATION AND CARCINOGENESIS IN RAT LIVER

Rabes, H. M., Jantsch, B., Tuczek, H. V., Riess, H., Galosi, A., Ludwig Maximilians Universitat Munchen, Inst. of Pathology, *Thalkirchner Str. 36, D8000 Munich 2, Bayern, Federal Republic of Germany*

Using enzyme histochemical, autoradiographic, biochemical and tissue culture methods the role of cell proliferation in N-nitroso-compound-induced liver carcinogenesis of the rat is studied. Preneoplastic clones are investigated with regard to their proliferative activity. Alkylation and repair of DNA as related to specific cell cycle periods is determined in a synchronized liver cell system in vivo as well as in perfused cell cycle-synchronized rat liver in vitro.

15. IN VIVO DNA DAMAGE/ALKALINE ELUTION ASSAY AS SHORT-TERM PRELIMINARY TEST FOR CHEMICAL CARCINOGENS

Brambilla, G., Cavanna, M., Parodi, S., Universita degli Studi di Genova, Dept. of Pharmacology, *Genoa, Italy*

OBJECTIVE: To evaluate, in animals treated with various non-carcinogens, procarcinogens and ultimate carcinogens, if the alkaline elution of DNA (Kohn et al., Biochemistry 15:4629, 1976) from cells of different organs, followed by microfluorometric determination of DNA in the eluted fractions, gives results positively correlated with the tumorigenic activity of these compounds; thus, to determine whether this technique, which allows metabolic activation in vivo and provides information on the organotropism of DNA-damaging activity, may play a useful role in the prescreening of chemical carcinogens.

APPROACH: This new procedure for the size-distribution analysis of DNA single-strands in cells from animals treated with a carcinogen is schematically as follows: 1) administration to the animal of a single (or few repeated) dose of the compound; 2) preparation after a variable period of time of cell suspensions from the supposed target organs; 3) fractionating of a DNA single-strand according to its size by alkaline elution of cellular lysates on Millipore filter; 4) microfluorometric determination of DNA in the eluted fractions by a modification of the method of Kissane and Robins (J. Biol. Chem. 233:184, 1958). The procedure does not require the prelabeling of DNA (which is tedious and/or problematic in organs devoid of cells in proliferating activity), provides, because of the greater number of cells that can be routinely processed (1 to 2 times 10 to the sixth power instead of 2 to 5 times 10 to the fourth power), a higher and more constant recovery of DNA in the various fractions than do alkaline sucrose gradients, and makes possible, owing to the absence of sucrose, a more rapid and easier microfluorometric determination of DNA.

PROGRESS: Elution profiles obtained with liver cells from mice prelabeled with methyl-3H-thymidine were practically superimposable with those obtained with microfluorometric DNA dosage. The DNA damage induced in vivo with a single dose of N-nitrosodimethylamine, N-methyl-N-nitrosourea, 1,2-dimethylhydrazine (DMH), cycasin and N-diazoacetylglycine amide has been detected in different organs of mice or rats. The results obtained are rather consistent with the organotropism of these carcinogens, and show a satisfactory dose-dependence of DNA damage. (Text Abridged.)

16. QUANTITATIVE ALKYLATION OF NUCLEIC ACIDS BY CARCINOGENS

Shank, R. C., University of California, Irvine Campus, California College of Medicine, Dept. of Community & Environmental Medicine, *Irvine, California*, 92664, United States of America

The hypothesis that N-nitroso compounds are carcinogenic because they are converted to direct alkylating agents by only target tissues is attractive, but most studies designed to test the hypothesis have investigated formation of alkylated bases only after a single administration of a near lethal dose of carcinogen

which may be pharmacokinetically unrelated to environmental exposures. Proposed studies will continue to examine quantitatively the chemical nature and extent of alkylation of DNA in liver, kidney, lung, pancreas, and colon by five carcinogens which are converted to alkylating agents at widely different rates by these tissues. Sensitivities of these tissues to carcinogenic activities of 1,2-dimethylhydrazine, diethylnitrosamine, N-nitrosopyrrolidine, N-nitrosomorpholine, and methylnitrosourea are established and are being used as comparators with site of alkylation. Adult rats and hamsters will be given single doses of diethylnitrosamine ranging from 1/16 to 1 LD50 and ethylation of DNA will be quantitated over the following 216 hrs. A similar study will be conducted with methylnitrosourea in guinea pigs. Also, ethylation of DNA during chronic administration of low levels of diethylnitrosamine to rats will be measured in target and non-target tissue. Isolation, silylation, and gas chromatography-mass spectrometry analysis of newly detected DNA adducts in N-nitrosopyrrolidine- and N-nitrosomorpholine-treated rats will be pursued.

17. INTERACTIONS OF ALKYLATING AGENTS AND NUCLEIC ACIDS

Chang, C., Purdue University, School of Pharmacy & Pharmacol. Sciences, Dept. of Medicinal Chemistry & Pharmacognosy, *Life Sciences Bldg., West Lafayette, Indiana,* 47907, United States of America

A detailed design of stable-isotope techniques, NMR spectroscopy and mass spectrometry, for studying drug-biochemical receptor interactions in vitro is proposed in this project. The main emphasis is on unraveling the bio-organic mechanism of actions of alkylating agents in terms of their reactions with RNA and DNA. The agents include methyl methanesulfonate, dimethyl sulfate, 1-methyl-3-nitro-1-nitrosoguanidine, 1-methyl-1-nitrosourea and their ethyl analogs, dimethylnitrosamine (activated by microsomal enzymes), alpha-acetoxydimethylnitrosamine, ethylene oxide, aziridine, 2-chloroethylamine, bis-(2-chloroethyl)amine, bis-(2-chloroethyl)methylamine, 1,3-bis(2-chloroethyl)-1-nitrosourea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, 2-chloroethyl isocyanate, cyclohexyl isocyanate. The reactions of both natural abundance and 13C-and 2H-enriched alkylating agents with mononucleotides, dinucleotides, RNA and DNA will be carried out in aqueous solution and maintained at neutrality by a pH-Stat, and the products will be unequivocally characterized by UV, IR, NMR and mass spectral analysis. The conformational variations will be determined by 1H-1H, 13C-1H, 31P-1H and 13C-31P coupling analysis. The relative chemical kinetics will be investigated by NMR using stable-isotope enriched agents. The alkylated product distribution will be calculated from NMR integration curves, and mass spectrometry using either GC-MS method or internal reference method. The reactive sites, relative chemical kinetics, conformational variations, cytotoxicity of alkylated products and product distribution will be utilized to correlate the structure-activity relationship.

18. STUDIES OF DNA CHANGE INDUCED BY PANCREATIC CARCINOGENS

Longnecker, D. S., Yager, J. D., Curphey, T. J., Unger, J., Dartmouth College, School of Medicine, Dept. of Pathology, *P.O. Box 833, Hanover, New Hampshire,* 03755, United States of America

The objective of this project is evaluation and comparison of damage of pancreatic DNA induced by several experimental carcinogens in rats. As specific parameters of damage are defined in the course of working with DNA from whole pancreas, studies will be extended to compare DNA damage in acinar cells and duct cells, and to compare DNA damage in pancreas with that in several other tissues. The compounds which will be evaluated include azaserine, N-gamma-(N-methyl-N-nitrosocarbamoyl)-L-2,4 diaminobutyric acid, N-nitrosobis (hydroxypropyl) amine and agaritine. Technical approaches include synthesis of radioactive carcinogens, DNA alkylation studies (carcinogen binding), and identification of specific abnormal nucleosides.

19. MECHANISM OF MUTAGENESIS AND CARCINOGENESIS

Guttenplan, J. B., New York University, School of Dentistry, Dept. of Biochemistry, *421 1st Ave., New York, New York,* 10010, United States of America

N-nitroso compounds comprise one of the most versatile and potent classes of carcinogens. As they are present in or formed from many environmental sources they are suspected of contributing to the etiology of human cancer. The alkylating efficiencies of and product distributions produced by N-nitroso compounds may vary between N-nitroso compounds and thus affect their mutagenic potencies. In addition, cellular reponses to alkylation may enhance or attenuate the effects of alkylation. These responses are not so well understood and are dose-dependent. This grant proposal addresses itself to the problems of relative alkylation and mutation inducing efficiencies of N-nitroso compounds and the biological responses to alkylation by N-nitroso compounds at different dose levels. The main areas of investigation in the project are: 1. Measurement of mutagenic potencies and rates of decomposition of N-nitroso compounds. These measurements will allow the expression of mutagenic potencies in terms of mutants per nanomole of mutagen metabolized, and will be carried out at many dose levels using Salmonella tester strains. 2. Investigations into the effects of bacterial strain, growth rate and growth phase on mutagenesis induced by N-nitroso compounds. These factors may influence contributions to mutagenesis attributable to differing DNA repair systems or different extents of DNA repair. 3. Investigations into the effectiveness of subthreshold doses of N-nitroso compounds in enhancing mutagenesis induced by other N-nitroso compounds and other classes of mutagens (comutagenesis). 4. Investigations into the relationship between O6-alkylation of bacterial DNA by N-nitroso compounds and mutagenesis induced by N-nitroso compounds. Effects of dose-levels, bacterial strains and bacterial growth rates will also be investigated. 5. Effects of inhibitors of error-prone DNA repair on mutagenesis induced by N-nitroso compounds, in several bacterial strains.

20. ALTERATION OF NAD METABOLISM BY CHEMICAL CARCINOGENS

Jacobson, M. K., North Texas State University, School of Arts & Sciences, Dept. of Chemistry, *N. Texas Station, Box 13707, Denton, Texas,* 76203, United States of America

The pyridine nucleotide NAD is directly involved in the regulation of cell growth. We have observed that carcinogenic N-nitroso compounds cause a rapid depression of cellular NAD. This depression appears to be related to NAD-dependent nuclear modification reactions associated with carcinogen-induced damage to DNA and/or repair of DNA damage. A major objective of this proposal is to study in detail the ability of N-nitroso compounds of known carcinogenicity to cause acute depression of NAD in 3T3 cells and in mitogen stimulated human lymphocytes. Several classes of direct-acting and indirect-acting carcinogens will be examined to determine whether acute depression of NAD is a general effect of carcinogens. The extent of DNA damage caused by N-nitroso compounds and the rate of DNA repair of carcinogen induced DNA damage will be studied in normal and NAD depleted 3T3 cells. Sensitive, quantitative methods involving gas chromatography-mass spectrometry and high pressure liquid chromatography for measuring NAD-dependent nuclear modifications as mono- and poly-adenosine diphosphoribose (ADPR) will be developed. The extent of these NAD-dependent nuclear modifications will be studied in normal cells and in cells exposed to carcinogenic N-nitroso compounds. Carcinogen-induced DNA damage and the rate of DNA repair will be studied in the presence of the methylated xanthines caffeine and theophylline which are specific inhibitors of NAD-dependent modifications.

II. OTHER BIOCHEMICAL AND IMMUNOLOGICAL EFFECTS OF NITROSO COMPOUNDS

21. CONTROL OF PROTEIN METABOLISM AND GROWTH OF NORMAL AND CARCINOGEN-TRANSFORMED RAT LIVER CELLS

Seglen, P. O., Solheim, A., Radiumhospitalet, Tissue Culture, *Montebello, Oslo 3, Norway*

OBJECTIVE: Identification of the substances and mechanisms which control general protein synthesis and degradation, i.e., growth, in normal rat hepatocytes and elucidation of how

these control functions are altered in cells malignantly transformed by chemical carcinogens.

APPROACH: Isolated hepatocytes are prepared by collagenase perfusion from the livers of normal rats, and from rats treated with a chemical carcinogen (diethylnitrosamine) in vivo. The capacity of such cells for protein synthesis and degradation is measured by isotope (14C-valine) incorporation and release, and by assessment of the overall nitrogen balance (analysis of changes in amino acids, urea and ammonia). The effects of various physical and chemical agents (hormones, sera, metabolites, ions and gasses, surface contact, etc.) on these parameters will be tested at various stages of in vivo carcinogenesis and at various times of in vitro culture. In vitro carcinogenesis may also be attempted. A major aspect of the project is the study of the biochemistry of growth, in particular the interrelationships between protein synthesis and degradation, intracellular amino acid pools and amino acid transport.

PROGRESS: Isolated hepatocytes synthesize protein actively. Amino acids for protein synthesis are taken from an intracellular pool, but the total intracellular amino acid content is compartmented into several pools. Despite their protein synthetic activity, hepatocytes are in negative nitrogen balance because their rate of protein degradation exceeds that of protein synthesis. Ammonia, either generated metabolically during incubation in a tissue culture medium, or added as ammonium chloride, inhibits protein degradation and thus improves the nitrogen balance. Ammonia inhibits lysosomal function by accumulating within the lysosomes and has therefore become a very useful tool in the study of lysosomal protein degradation.

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22. IN VITRO DIFFERENTIATION OF CLONAL CELLS

Sueoka, N., University of Colorado, Boulder Campus, School of Arts & Sciences, Dept. of Molecular, Cellular & Developmental Biology, *1200 University Ave., Boulder, Colorado,* 80302, United States of America

We are studying in vitro differentiation of different cell types from ethylnitrosourea-induced rat neurotumors. Biochemical cytological characterization of several cell lines clonally isolated from a rat neurotumor (RT4) is conducted on their neuronal and glial properties. We are particularly interested in the phenomenon of 'cell-type conversion' that we have discovered, in which a multipotential stem cell line converts to three different cell types in culture and these derivative cell types possess either glial (S100) or neuronal (voltage-dependent Na ion influx) properties. Population-wise, the stem cells have both properties. Several known glial and neuronal properties will be examined in these cell lines. Cytochemical analyses of the individual cells of the stem-cell population will be made. Various culture conditions that may influence the rate and direction of cell-type conversion will be examined. Another phenomenon we have noticed is 'conversion coupling,' in which a specific combination of two or more differentiated characteristics are expressed in a stem cell-derived cell type. This is extremely important for the in vitro studies of cell differentiation and will be studied in detail. Cloning of a few additional neurotumors of central and peripheral nerve origins will also be performed to establish new families of neurocell types which are related by cell-type conversion of stem cells.

23. BINDING OF METHYL-NITROSOUREA IN PANCREATIC TISSUE

Woolley, P. V., Pinsky, S. D., Georgetown University, School of Medicine, Dept. of Medicine, *3800 Reservoir Rd. N.W., Washington, District of Columbia,* 20007, United States of America

This project examines in detail the sites of binding of two important carcinogens, 1-methyl-1-nitrosourea (MNU) and 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) to pancreatic tissue. Emphasis is placed on modification of components of the cell nucleus, both DNA and proteins (histones and non-histones). Both of the above compounds can react with macromolecules by two chemical routes, namely alkylation (MNU and MNNG) and carbamoylation (MNU) or guanidination (MNNG), so that a total of four chemical processes are to be examined. Results to date in a model system using L1210 cells in suspension culture indicate that both compounds modify histones and non-histones. The guanidination of histones by MNNG is quantitatively the most important. Histones H1, H2b and H3 are primary targets while H4 is less modified. Non-histones also undergo modification. In guinea pigs both MNU and MNNG show relatively low uptake in pancreas and stomach compared to liver, kidney and small intestine following intraperitoneal injection. However, there is a high concentration of the carcinogens in nuclei of pancreas and stomach relative to the other organs. The significance of these reactions in modifying living tissue in the GI tract, especially the pancreas, in the guinea pig, is currently under investigation. The purpose of the study is to define reactions of significance in the production of pancreatic cancer.

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24. RIBONUCLEOPROTEIN COMPLEXES IN RENAL CARCINOMA

Irwin, D., George Washington University, School of Medicine & Health Sciences, Dept. of Biochemistry, *2121 Eye St. N.W., Washington, District of Columbia,* 20037, United States of America

The equilibrium between unassociated (ribosome-free) cytoplasmic messenger ribonucleoprotein (mRNP) and polysome-associated mRNP is likely to be a key step in the translational control of cell proliferation. Therefore, the relative amounts and kinetics of these classes of mRNP and their nuclear precursors (hnRNP) will be studied in an organ (the kidney) that can be made to show graded responses from hypertrophy to hyperplasia to neoplasia. Renal hypertrophy will be produced by contralateral nephrectomy, hyperplasia by injection of folic acid, and neoplasia by dimethylnitrosamine treatment of Charles River mice. Particular attention will be paid to homologies in the protein components of the RNPs as assessed by labeling and by two-dimensional gel electrophoresis and the kinetics of labeling the integral RNA which may indicate precursor-product relationships. These proteins may stabilize the RNPs during stimulated growth and modulate the processing and translation of their RNAs; other work in this laboratory strongly suggests that the rate of degradation of protein and ribosomal RNA, rather than the rate of synthesis, is a major controlling mechanism in renal growth. The relative efficiencies of isolated RNP complexes in total protein synthesis will be measured in a cell-free protein synthesizing system and compared to that of deproteinized poly(A)-containing mRNA and reconstituted RNP systems. We propose to show that RNP complexes isolated during different patterns of growth contain some differences in their protein complements, that these differences are directly related to the control of mRNA expression and that these proteins determine the distribution of mRNA between translating and non-translating states.

25. CYCLIC NUCLEOTIDES IN COLONIC CARCINOGENESIS

DeRubertis, F. R., Craven, P. A., Saito, R., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Dept. of Medicine, *University Dr. C, Pittsburgh, Pennsylvania,* 15240, United States of America

Studies are in progress to assess the roles of the cyclic nucleotides adenosine 3'5'-monophosphate (cAMP) and guanosine 3'5' monophosphate (cGMP) in colonic carcinogenesis. Rapid activation of the guanylate cyclase (GC)-cGMP system of colonic mucosa of several species including man was previously demonstrated in response to the colon carcinogens N'-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and N-methylnitrosourea. Recent results indicate that colonic GC is also activated during oxidation of the carcinogen 4-acetyl-aminobiphenyl. Data from several sources now implicate redox or free radical reactions as a general mechanism of GC activation, and indicate that this enzyme may be a target for interaction with reactive forms of several carcinogens. The precise mechanism of

carcinogen activation of colonic GC, the ability of reactive derivatives of 1,2 dimethylhydrazine (DMH) to stimulate the enzyme and the biologic actions of MNNG induced increases in colonic cGMP are under study. In related work comparing cyclic nucleotide metabolism in superficial and crypt cells from rat colonic epithelium and the effects of DMH administration on this metabolism, a DMH induced alteration in the isoenzyme distribution of soluble cAMP-dependent protein kinase activity (PK) has been identified. In both superficial and crypt cells from control epithelium the type II isoenzyme form of PK dominates (greater than 85% of total PK), whereas 3 days after DMH approximately 50% of PK is the type I form in both cell populations. The consequences of this shift with respect to the ability of cAMP to suppress DNA synthesis, as it does in normal epithelium, are under study.

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III. METABOLISM AND PHARMACOLOGY OF NITROSO COMPOUNDS

26. CHEMICAL CARCINOGENS IN THE PANCREAS

Epstein, S. S., University of Illinois, Medical Center at Chicago, School of Public Health, Dept. of Occupational & Environmental Medicine, *Box 4348, Chicago, Illinois*, 60680, United States of America

Studies are proposed to further investigate the in vivo metabolism of benzo(a)pyrene in the pancreas, with particular reference to characterization of its metabolites and cellular distribution. Studies are also proposed to investigate the uptake, in vivo metabolism and excretion of di-isopropanolnitrosamine and 4-nitroquinoline-1-oxide and their metabolites by the pancreas from control and induced animals.

27. CHEMOTHERAPEUTIC AND CARCINOGENIC METHYLATING AGENTS

Weinkam, R. J., Purdue University, School of Pharmacy & Pharmacol. Sciences, Dept. of Medicinal Chemistry, *Life Sciences Bldg., West Lafayette, Indiana*, 47907, United States of America

Active intermediates formed in the metabolic activation of procarbazine will be investigated using synthesized metabolites and deuterium labeled variants. Activation pathways will be related to the carcinogen 1, 2-dimethylhydrazine. Metabolism of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DIC) and the closely related carcinogen 3,3-dimethyl-1-phenyltriazene will be studied in vitro and in vivo using high performance liquid chromatography and chemical ionization mass spectrometry techniques. Nucleophilic susceptibility and proton exchange of the methylating species generated from these compounds and dimethyl-N-nitrosamine and N-methyl-N-nitrosoures will be compared to determine whether identical intermediates have been generated. The diverse biological effects of these agents may be related to different properties or distribution of the respective chemically activated precursors to the ultimate methylating agent. The half-life of these metabolically generated precursors will be measured.

28. ACTIVATION AND REACTIVITY OF NITROHETEROCYCLES

Olive, P. L., Johns Hopkins University, School of Medicine, Dept. of Oncology, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

Humans are exposed to nitroheterocyclic compounds through extensive use in medicine and industry; these chemicals are metabolically 'activated' to toxic, DNA damaging and, in many cases, tumorigenic agents. We will attempt to determine the relationship between drug 'activation,' DNA damage, mutagenicity and transforming ability of selected nitroheterocycles. Specifically, we will determine whether the electronegativity (reactivity of these chemicals) as reported in the literature, is correlated with their ability to damage DNA, as measured by hydroxylapatite chromatography, their mutagenicity in bacterial tester strains, or their ability to transform cultured C3H/10T1/2 mammalian cells. In addition, we intend to determine whether the amount of nitrofuran-induced DNA damage or its rate of repair differs in specific target organs for tumor production, and further, whether this damage is due solely to metabolic activation in the target tissue or to tranfer of 'activated' intermediates of nitrofuran metabolism.

29. CARCINOGENESIS STUDIES IN CULTURED COLON FROM EXPERIMENTAL ANIMALS AND HUMANS

Autrup, H., Harris, C., Stoner, G., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Human Tissue Studies Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

A model system for studying chemical carcinogenesis in colon has been developed. Human colon can generally be maintained 7-24 days and rat colon at least 63 days as explant cultures. Cultured human colon and rat colon have the ability to enzymatically convert various classes of chemical carcinogens, such as polycyclic aromatic hydrocarbons, N-nitrosamines, mycotoxins and hydrazines, into metabolites which react with cellular macromolecules, such as DNA and protein. A 100-fold interindividual variation in the binding of benzo(a)pyrene (BP) and a 60-fold variation in the binding of 1,2-dimethylhydrazine (DMH) to DNA were found. A positive correlation in the binding level of BP and DMH to DNA was observed. The carcinogen-DNA adducts for aflatoxin B1, BP, N-nitrosodimethylamine and DMH have been identified, and were qualitatively similar in both rat and human colon.

BIBLIOGRAPHIC REFERENCES: Autrup, H., Essigmann, J.M., Croy, B.G., Trump, B.F., Wogan, G.N., and Harris, C.C. Metabolism of aflatoxin B1 by cultured human tissues. Cancer Res. 39: 694-698, 1979. Autrup, H. Water-soluble BP-conjugates formed by cultured human colon. Biochem. Pharmacol. 28: 1727 1730, 1979.

30. METABOLISM OF CHEMICAL CARCINOGENS BY CULTURED ESOPHAGUS, BRONCHUS AND PANCREATIC DUCT FROM HUMANS AND EXPERIMENTAL ANIMALS

Harris, C., Autrup, H., Stoner, G., Connor, R., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Human Tissue Studies Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Human bronchus, pancreatic duct and esophagus can be cultured for several weeks in a chemically defined medium. This controlled experimental setting provides an excellent in vitro system to study the metabolism of chemical carcinogens including those found in tobacco smoke and the environment. Several classes of chemical carcinogens, polynuclear aromatic hydrocarbons, N-nitrosamines, hydrazines, and mycotoxins can be metabolically activated by human tissues.

The metabolic pathways leading to the formation of DNA adducts in cultured bronchus and esophagus have been defined for benzo(a)pyrene, aflatoxin B1, and N-nitrosodimethylamine. The pathways to organic-extractable and water-soluble metabolites of benzo(a)pyrene were qualitatively similar in all 3 tissues. The adducts between these carcinogens and DNA in human bronchus and esophagus are essentially the same as those found in experimental animals in which the chemicals are carcinogenic. When different people are compared a marked variation in binding levels is found e.g., the interindivdual variation in the binding of BP to DNA in cultured human bronchus was 75-fold (100 patients). A variation in carcinogen binding levels in different target tissues within an individual was also found.

BIBLIOGRAPHIC REFERENCES: Harris, C., Autrup, H., and Stoner, G.: Metabolism of benzo(a)pyrene by cultured human tissues and cells. In: Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment, Ts'o, P.O.P. and Gelboin, H.V. (Eds.), Academic Press, Vol. 2, pp. 331-342, 1978. Autrup, H., Eisingman, J.M., Croy, R., Trump, B.F., Wogan, G.N., Harris, C.C.: Metabolism of aflatoxin B1

and identification of aflatoxin B1-DNA adducts by cultured human bronchus and colon, Cancer Res., 39: 694-698, 1979.

31. CARCINOGENESIS STUDIES IN CULTURED RAT ESOPHAGUS

Stoner, G., Autrup, H., Harris, C. C., Katoh, Y., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Human Tissue Studies Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

A model system for the study of carcinogenesis in the rat esophagus has been developed. Explants of rat esophagus have been cultured in a chemically defined medium for a period of 50 days. Using immunoperoxidase techniques, rat esophageal keratin can be stained with antibody to mouse skin keratin, thus providing an immunological marker of epithelial cell differentiation. The metabolism of a series of N-nitrosamine carcinogens is being investigated in rat esophagus. It is anticipated that this model system will be useful for investigations of chemical carcinogenesis in the rat esophagus.

32. DIALLYLNITROSAMINE CARCINOGENESIS - SPECIES DIFFERENCES

Grandjean, C. J., Gold, B., Pour, P., University of Nebraska, Medical Center, School of Medicine, Eppley Institute, *42nd & Dewey Aves., Omaha, Nebraska*, 68105, United States of America

In general nitrosamines constitute a class of potent carcinogens that induce tumors in all rodent species, although the tumor sites do vary between species. There are only a few examples of non-carcinogenic nitrosamines that are not fully substituted at the alpha-positions and hence prevent alpha-hydroxylation (activation). Diallylnitrosamine (DAN) is one such compound and was reported non-carcinogenic in the BD rat. However, we have found DAN to be a potent inducer of respiratory tract tumors in the Syrian golden hamster upon multiple or a single subcutaneous (s.c.) injection. These comparative findings give rise to multiple questions concerning this type of species difference never observed before with nitrosamines.

To determine some of the factors that may contribute to this dramatic species difference in light of the present knowledge about nitrosamine carcinogenesis the following studies are proposed: 1) Determination of the carcinogenic response of the Syrian golden hamster and the BD(IX) rat to DAN using intragastric and subcutaneous administration; 2) determining whether DAN is metabolically converted into other nitrosamines in either species using both i.g. and s.c. routes of administration; 3) determination of the qualitative and quantitative distribution of covalently bound adducts to nucleic acids in high and low risk tissues in the hamster and rat upon i.g. and s.c. administration of DAN-3H; 4) determination of the qualitative and quantitative persistence of specific alkylated components of nucleic acids in high and low risk tissues in both species, as determined on the basis of the proposed chronic animal testing.

A comparative species analysis of these results in terms of metabolism, tissue distribution, qualitative and quantitative nature of nucleic acid alkylation and the persistence of these alkylated products should give us a great deal of information and help understand the unique species difference observed with DAN which may be expanded to ultimately include other compounds. Also, these results will be of great value in understanding and perhaps expanding the present knowledge of nitrosamine carcinogenesis.

33. METABOLISM OF THE CARCINOGEN NITROSONORNICOTINE

Hecht, S. S., Hoffmann, D., Chen, C. B., Rivenson, A., American Health Foundation Inc., Section of Organic Chemistry, *320 E. 43rd St., New York, New York*, 10017, United States of America

The tobacco specific nitrosamines, N'-nitrosonornicotine (NNN), and 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) occur in unburned tobacco and mainstream and sidestream tobacco smoke in high concentrations and are carcinogenic in experimental animals. Therefore, these nitrosamines may be causative factors in tobacco related cancers, which have been estimated to comprise more than one-third of the cancers occurring in U.S. males. It is the objective of this proposal to determine the mechanism of action of these major environmental carcinogens through studies of metabolism and binding to cellular macromolecules and to establish the basis for their organ-specific effects. The structures of the NNN-DNA and NNK-DNA adducts formed after administration to rats or hamsters of specifically labelled NNN or NNK will be determined using chromatographic and spectroscopic methods, as well as synthetic marker compounds. The formation and persistence of these adducts, as well as the rates of activation and detoxification in target and non-target organs will be measured. The effects of potential chemopreventive agents on the carcinogenicity of NNN will be determined through metabolic studies and bioassays in rats. The metabolism of NNN and NNK in smokers will also be investigated. These goals are important for a better understanding of the tobacco related cancers and the mechanisms of nitrosamine carcinogenesis.

34. ENVIRONMENTAL NITROSAMINES - METABOLISM AND CARCINOGENESIS

Hecht, S. S., Hoffmann, D., American Health Foundation Inc., Division of Environmental Carcinogenesis, *Hammond House Rd., Valhalla, New York*, 10595, United States of America

The metabolism and mechanisms of activation of the environmental carcinogens N-nitrosopyrrolidine, N-nitrosomorpholine, and N-nitrosodiethanolamine are being investigated. These studies are focused on the identification of electrophilic metabolites and of carcinogen DNA adducts derived from the three nitrosamines. The DNA adducts are thought to arise from metabolic alpha-hydroxylation. This hypothesis is being tested by comparison of synthetic marker compounds with those formed in vitro and in vivo during carcinogen-DNA binding. The effects on metabolism and activation of environmental modifiers and potential inhibitors of carcinogenesis are also being studied.

BIBLIOGRAPHIC REFERENCES: S.S. Hecht, C.B. Chen, G.D. McCoy, D. Hoffmann and L. Domellof. Alpha-Hydroxylation of N-nitrosopyrrolidine and N'-Nitrosonornicotine by Human Liver Microsomes. Cancer Letters 8, 35-41 (1979). C. B. Chen, S.S. Hecht, D. Lin, G.D. McCoy and D. Hoffmann. Assays for Metabolic alpha-Hydroxylation of N'-Nitrosonornicotine and N'-Nitrosopyrrolidine and the Influence of Modifying Factors. International Agency for Research on Cancer Scientific Publications, 1980 In Press.

35. ABSORPTION OF CHEMICAL CARCINOGENS IN COLON

Rose, R. C., Nahrwold, D. L., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Physiology/Surgery, *500 University Dr., Hershey, Pennsylvania*, 17033, United States of America

The aim of this study is to gather information about the absorption of known chemical carcinogens by epithelia of the mammalian colon.

An extensive literature exists concerning the intracellular actions of chemical carcinogens in producing cancer of gastrointestinal epithelia and other tissues. Little is known, however, about the absorption of carcinogens from the intestinal lumen into the lining epithelia, or the absorption of carcinogens from the lumen into the blood. The primary carcinogens of interest will be 2-acetylaminofluorene; 2', 3-dimethyl-4-aminobiphenyl; N-methyl-N'-nitro-N-nitrosoguanidine; and 1,2-dimethylhydrazine. We propose to determine with reliable in vitro and in vivo techniques the following: 1) The mechanism of transport in the colon of four known carcinogens. Studies will be performed in vivo and in vitro to determine if net absorption or secretion takes place. Appropriate studies will indicate whether each carcinogen crosses the colonic mucosa by simple diffusion, facilitated transport, or some form of active transport. 2) The effects of bile acids and fatty acids on the transport of carcinogens across colon and into colon mucosal cells. This aim is specifically promoted in the Announcement of the Continuation of the National Large Bowel Cancer Project, FY 78 I.C. (10); page 5. Guinea pigs and rats will be the primary subjects of investigation. Some in vitro studies will be performed on human tissue removed at the time of surgery for purposes of comparison. Short of limiting carcinogens from the environment the most effective measure for reducing the incidence of cancer will be to limit entry of these substances into the body tissues. The information we propose to gather may serve as a base from which to design experiments on specifically limiting gastrointestinal absorption of chemical carcinogens. Thus, results of the proposed

project might help us to understand, and begin a new approach to controlling environmental carcinogenesis associated with dietary factors.

36. FORMATION AND METABOLISM OF N-NITROSO COMPOUNDS

Magee, P. N., Temple University, School of Medicine, Dept. of Pathology, *3322 Broad St., Philadelphia, Pennsylvania*, 19122, United States of America

The metabolism, including absorption, distribution in the body, interaction with cellular constituents, and excretion, of a number of selected N-nitroso compounds and their metabolic products will be studied in rats and other species. The N-nitroso compounds will include nitrosamines, nitrosamides and nitrosamidines as well as methylhydrazines and phenyldimethyl-triazene. Most of the compounds chosen are carcinogenic but some are reported to be noncarcinogenic. Emphasis will be placed on nitrosamides that are used in clinical human cancer chemotherapy since these are the only N-nitroso compounds that are deliberately administered to human subjects. The compounds will be variously labeled with radioactive (14C and 3H) and stable (15N and 13C) isotopes. Nitrosamines will be measured in tissues and body fluids by a new method of differential pulse polarography that is currently being developed in these laboratories. The metabolism of secondary amines that occur normally in the body, including dimethylamine, pyrrolidine, piperidine, proline and sarcosine, will be studied in relation to their potential endogenous nitrosation under various conditions. The metabolism of nitrate and nitrite will be studied since relatively little is known in this area. Nitrite estimations in tissues and body fluids will be carried out by a new method based on nitrosation of diphenylamine. Nitrite and nitrate labeled with 15N will be used in part of this work. The development and improvement of the new method for nitrosamine and nitrite determination will be a major part of the proposed research. It is hoped that the proposed research will increase knowledge of the role of alkylation reactions in carcinogenesis by nitroso compounds and the possible role that these compounds may play in the causation of animal and human cancer.

37. MUTAGENIC ACTIVITY OF NITROSAMINES IN CHEMICAL MODELS (RATS)

Tannenbaum, S. R., Massachusetts Inst. of Technology, School of Science, Dept. of Nutrition & Food Science, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

This is part of a broader project. A summary of this subproject is not available.

IV. CELL TRANSFORMATION BY NITROSO COMPOUNDS AND RELATED IN VITRO STUDIES

38. THE ROLE OF PROMOTERS IN TWO-STEP CARCINOGENESIS

Kinsella, A. R., Radman, M., Paterson Lab., *550 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom*

OBJECTIVE: To attempt to correlate aberrant chromosomal segregation events with transformation following chemical carcinogen treatment, by the use of known inhibitors of promotion and anticarcinogenic agents.

APPROACH: V79 Chinese hamster lung fibroblasts are treated either with carcinogen alone or simultaneously with the carcinogen and inhibitor, in the presence of 3 times 10 to the minus 5th power M BUdR for twenty-seven hours or two cell cycles. A comparison is then made of the sister chromatid exchange (SCE) frequencies and aberration frequencies in the presence or absence of the inhibitor.

In addition, these studies are being correlated with the effects of inhibitors of promotion on chemically induced transformation, in the C3H 10T 1/2 mouse transformation system.

PROGRESS: Antipain, an anti-protease and inhibitor of promotion inhibits N-methyl-N'-nitro-N-nitrosoguanindine (MNNG)-induced chromosome aberrations and inhibits MNNG-induced transformation (Kinsella and Radman, in press).

39. BIOLOGICAL PROPERTIES OF FETAL BRAIN CELLS DURING MALIGNANT TRANSFORMATION IN CELL CULTURE

Laerum, O. D., Haugen, A., Mork, S., Moehle, B., Dahl, O., Universitet i Bergen, Gade Inst., Dept. of Pathology, *5016 Haukeland Hospital, 5000 Bergen, Norway*

OBJECTIVES: a) Investigation of phenotypic properties of fetal rat brain cells during the gradual malignant transformation in cell culture. b) Use of malignant neurogenic cell lines for experimental therapy.

APPROACH: The project was started in Professor M. F. Rajewsky's group in Tubigen and is continued in close cooperation with his institute in Essen, Germany. Pregnant rats, 18th day of gestation, are given a single pulse of ethylnitrosourea, which gives a very high yield of neurogenic tumors in the offspring. Shortly after the in vivo carcinogen pulse, the brains of the fetuses are transferred to cell culture, where they undergo gradual malignant transformation until they are tumorigenic after a period of 200 days. The differentiation of neoplastic and pre-neoplastic cells is studied using different nervous system specific markers. Karyotyping and measurement of cellular DNA content are performed in parallel.

PROGRESS: The experimental system has been reported on earlier and results from detailed electron microscopic analysis of the same sequence of transformation are published in the same journal. Different classes of neural cells which participate in the process of neoplastic transformation have been identified by transmission electron microscopy. Concomitant alterations of cytoskeleton have been investigated in parallel. Expression of different nervous system specific markers is under investigation.

Cytostatic effects of different platinum compounds on such neoplastic cell lines have been investigated under in vivo and in vitro conditions. Combination therapy with cytostatic drugs and hypothermia is in progress.

40. ANCHORAGE CONTROL OF PROTEIN METABOLISM AND GROWTH IN RAT HEPATOCYTES DURING CHEMICALLY INDUCED LIVER CARCINOGENESIS

Gjessing, R., Seglen, P. O., Norsk Hydro's Inst. for Cancer Research, *Montebello, Oslo 3, Norway*

OBJECTIVE: Investigation of the basic mechanisms of cell-to-substratum anchorage on different kinds of substratum, both for normal and chemically transformed rat liver cells, and of the effects of cell-anchorage on general protein metabolism, i.e., growth, of hepatocytes in different stages of chemically induced liver carcinogenesis.

APPROACH: Isolated hepatocytes are prepared by collagenase perfusion (P.O. Seglen, Methods in Cell Biology 13, 29-83, 1976) from the livers of normal rats, and from rats treated with a chemical carcinogen (diethylnitrosamine) in vivo. Tissue culture dishes are pretreated with various cell-binding proteins to produce attachment substrata of different kinds, and the characteristics of hepatocyte anchorage to each type of substrate are studied, including the effects of anchorage on growth, protein metabolism and other metabolic processes. An attempt will also be made to select preneoplastic hepatocytes on the basis of differential substratum attachment. Growth will be measured as net protein accumulation by isotope incorporation and release.

PROGRESS: The basic requirements for rat hepatocyte anchorage to a substrate of adsorbed serum protein have been determined (J. Cell. Sci. 34, 117-131, 1978) and similar studies on anchorage to substrata of activated polystyrene (tissue culture dishes), collagen, asialofetuin, poly-l-lysine, concanavalin A and albumin have been conducted.

41. ALKYLATING CARCINOGEN MUTAGENESIS IN MAMMALIAN CELLS

Burki, H. J., University of California, Berkeley Campus, Lawrence Berkeley Lab., Biology & Medicine Division, *Berkeley, California*, 94720, United States of America

It is proposed to develop a model system for studying the mutagenesis of alkylating carcinogens in asynchronous and synchronous mammalian cells in vitro. Using asynchronous cells of different species and tissue origin, we will measure the production of mutations by ethylnitrosourea (ENU) and related environmental carcinogens. We will determine the effects of cellular repair processes on the induction of mutations by measuring the

loss of carcinogen-produced damage and relating it to mutagenesis. In addition, we will measure the induced frequency of certain specific mutations during the cell cycle to test if specific mutagenic changes are associated with specific cell-cycle times.

ENU is used because of its advantageous chemical characteristics, and because of its special carcinogenic properties. Exposing homogeneous populations of selectively synchronized mammalian cells to this agent offers a novel way to test for alkylation-induced mutations in mammalian cells. These experiments may give us a practical understanding of chemical mutagenesis from alkylating agents which will be useful for estimating total risks, as well as risks relative to other environmental carcinogens, to the human population.

42. CARCINOGENESIS OF PANCREATIC DUCTAL TISSUE IN VITRO

Githens, S., Holmquist, D. R., Ruby, J. R., Louisiana State University, University of New Orleans, School of Science, Dept. of Biological Sciences, *Lakeshore Dr., Lake Front, New Orleans, Louisiana*, 70122, United States of America

Pancreatic ducts of the rat and hamster will be isolated and cultured for six to eight weeks in an agarose matrix in CMRL-1066 supplemented with fetal bovine serum, insulin, and dexamethasone. The ducts will be exposed to benzo(a)pyrene, 7,12-dimethylbenzanthracene, and N-nitroso-bis(2-oxopropyl)amine in an effort to cause histologic changes similar to those seen in the pancreas in vivo. The effects of these carcinogens will be potentiated by exposure in the presence of liver microsomes, the use of younger animals with larger proportions of mitotically active cells, and by the addition of growth and tumor promoters such as phorbol esters to the growth medium. The effects of the carcinogens will be monitored morphologically with light and electron microscopes, biochemically by enzyme assays and SDS-polyacrylamide gel electrophoresis, and biologically by assessing the ability of the treated cells to form colonies in soft agar and to grow in a host animal.

BIBLIOGRAPHIC REFERENCES: Characterization of Ducts Isolated from the Pancreas of the Rat. Sherwood Githers III, Doris R. G. Holmquist, Joelle F. Whelan, and John R. Ruby. Journal of Cell Biology. Scheduled for April 1980. Morphologic Response of Cultured Rat Pancreatic Ducts to Carcinogens. D. R. G. Holmquist, J. F. Whelan, S. Githens, and J. R. Ruby. Laboratory Investigation 40: 24-25 (1979).

43. CELL TYPE/HOST ASSAY OF MUTAGEN-CARCINOGEN ACTIVITY

Yerganian, G., Northeastern University, Graduate School, *360 Huntington Ave., Boston, Massachusetts*, 02115, United States of America

The study of the sensitivity of sister chromatid exchanges (SCE) in response to mutagens will be extended to short-term peripheral lymphocyte cultures. The purpose is to assess the advantages which are likely to stem from this procedure with regard to monitoring chronic exposures of test animals to low levels of mutagen and the introduction of suspected co-factors. The feasibility of conducting mutagen and carcinogen assays on the same target cell population and the same animal is presented by the autoimmune-prone Armenian hamster, Cricetulus migratorius (2n equals 22). Unless exposed to nitroso compounds to induce angioimmunoblastic lymphadenopathy (AIL) and immunoblastic sarcoma (IBS), the Armenian hamster remains characteristically tumor-free throughout a lifespan of 1,000 days. This contrasts sharply with the Chinese hamster, Cricetulus griseus (2n equals 22) which fails to respond to MNU (70 mg/k body weight) when administered intravenously.

In addition, clinical and epidemiological considerations have now clearly associated therapeutic regimes and environmental exposures to pesticide as co-factors for the precipitous onset of clinical AIL/IBS, notably in segments of the human population with recognized autoimmune-related disorders. Animal studies will attempt to simulate recognized episodes associated with clinical AIL. Monitoring low-level chronicity tests comprised of threshold levels of two or more suspected co-factors can only be achieved by the adaptation of the peripheral lymphocyte for the sensitivity provided by the SCE test.

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44. MUTAGENESIS OF NITRAMINES VIS-A-VIS NITROSAMINES

Nagasawa, H. T., Shirota, F. N., University of Minnesota, Minneapolis Campus, School of Pharmacy, Dept. of Medicinal Chemistry, *Morrill Hall, Minneapolis, Minnesota*, 55455, United States of America

The mechanism by which dimethylnitramine elicits tumor formation in rodents is still unknown. While oxidative mechanisms may be considered, it is possible that a reductive mechanism may supervene in activating dimethylnitramine to carcinogenic metabolites. This possibility will be investigated by subjecting dimethylnitramine and certain other selected secondary and primary nitramines to the Ames mutagenicity assay against mutant strains of Salmonella typhimurium under conditions which favor the enzymatic reduction of the nitramines. The corresponding secondary nitrosamines will also be subjected to these tests under comparable conditions.

45. CHEMICAL CARCINOGENESIS - MAMMARY GLAND ORGAN CULTURE

Banerjee, M. R., University of Nebraska, Lincoln Campus, School of Arts & Sciences, Dept. of Life Sciences, *Lincoln, Nebraska*, 68508, United States of America

The objectives of the proposed research are 1) to further assess the tumor producing capabilities of 7,12-dimethylbenz(a)anthracene (DMBA) induced nodule-like alveolar lesions (NLAL) and 2) to characterize the hyperactive outgrowths derived from enzymatically dissociated epithelial cells of DMBA treated glands in a serum-free, hormone supplemented organ culture of the whole mammary gland of BALB/c female mice. A limited number (2/12) of NLALs have been found to produce hyperactive lobuloalveolar (LA) outgrowths and one has so far produced a mammary adenocarcinoma. In order to obtain a more reliable assessment of the tumor potential of the NLAL, a larger sample of explants will be analyzed by the mammary fat pad transplantation procedure. Explants of NLAL cells in tissue fragments and enzymatically dissociated epithelial cells will be used. Several hyperactive LA outgrowths derived, one from a NLAL explant and 6 from explants of dissociated cells of DMBA treated glands, are carried by serial transplantation into mammary fat pads. Some of these outgrowths have produced mammary tumors. These outgrowth lines will be characterized for tumor potential and hormone sensitivity with respect to growth and specific gene (casein) expression. Expression of the casein gene will be measured by molecular hybridization, using a specific complementary DNA to casein mRNA (cDNAcsn), synthesized in my laboratory. Hormone responses will be monitored in organ culture of the outgrowth in the inguinal mammary fat pad, cultivated in a hormone supplemented, chemically defined medium. Experiments have been outlined for testing two additional nonhydrocarbon oncogens, N-methylnitrosourea (NMU) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), with respect to their potential to induce 'transformation' of the epithelial and the stromal cells in culture of the whole mammary organ. The results should further the establishment of the usefulness of this singular culture model for studies on interactions between the carcinogens, hormones and different cell types under conditions of biological coexistence in the whole breast.

46. AN IN VITRO MODEL OF HUMAN PANCREAS CARCINOGENESIS

Parsa, I., State University of New York, Downstate Medical Center, School of Medicine, Dept. of Pathology, *450 Clarkson Ave., Brooklyn, New York*, 11203, United States of America

It is proposed to develop an in vitro model of human pancreas carcinogenesis for the study of cellular evolution leading to human pancreatic neoplasia. Investigation of the differential roles of various cell populations of the pancreas in the activation of chemical carcinogens, promotors and inhibitors of pancreas carcinogenesis will be carried out.

The efficacy of culture conditions will be evaluated based on the morphological (LM) and ultrastructural (EM) preservation of the pancreatic explants, and attempts to improve such conditions will be made. Immediate and long-term effects of dimethylnitrosamine (DMNA), methylnitrosourea (MNU) and methylnitrosoguanidine (MNG) on human pancreas explants in culture will be evaluated by light and electron microscopy. Biological behavior of tumors induced in vitro by these carcinogens will be tested in xenogeneic host.

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V. STUDIES OF SPECIFIC TYPES OF CANCERS INDUCED BY NITROSO COMPOUNDS

A. GASTROINTESTINAL SYSTEM CANCER INDUCED BY NITROSO COMPOUNDS

47. EXPERIMENTAL PRODUCTION OF GASTRIC CARCINOMA IN RATS BY ADMINISTRATION OF NITROSO-DERIVATIVE - COMPARISON WITH HUMAN GASTRIC CARCINOMA

Tanaka, N., Miyauchi, M., Ueno, T., Fujimura, S., Chiba Cancer Center Research Inst., Dept. of Pathology, *666-2, Nitona-Cho, Chiba 280, Chiba, Japan*

OBJECTIVE: To produce an animal model of gastric and intestinal carcinoma in rats, experimental study has been carried out by administration of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and N-ethyl-N'-nitro-N-nitroso-guanidine (ENNG) in drinking water. For morphogenetical analysis of early stages of carcinoma in the glandular stomach, especially of ulcus carcinoma as well as signet ring cell type carcinoma, various methods such as microphotometry to measure the nuclear DNA content, electron-microscopic and scan-electron-microscopic examination as well as transplantation into the nude mice are employed for this study. The early stage of adenocarcinoma in this series is really difficult to differentiate from atypical regeneration of glands, as seen in human material.

APPROACH: Young Wistar rats were administered MNNG and ENNG in drinking water, respectively for 30 and 20 weeks. Animals were periodically sacrificed and findings retrospectively evaluated using the procedures described above, besides routine histological examination.

PROGRESS: ENNG reveals the strongest affinity to the intestinal mucosa rather than the gastric mucosa as target organ. MMNG causes a high incidence of gastric carcinomas more frequently, nearly 100% after 50 weeks. It is occasionaly extremely difficult to differentiate the early stage of adenocarcinoma in this series from atypical regeneration, often seen in human material, adenomatous polyp and/or atypical regeneration after marked metaplastic gastritis, even using the data accumulated by microphotometry.

48. EXPERIMENTAL INDUCTION OF INTESTINAL METAPLASIA

Matsukura, N., Kawachi, T., Sugimura, T., Ministry of Health & Welfare, Biochemistry Div., *5-1-1 Tsukiji, Chuo-ku, Tokyo, Tokyo, Japan*

OBJECTIVE: Intestinal metaplasia is often associated with human stomach cancer and is considered to be a precancerous lesion. The relationship between stomach cancer and intestinal metaplasia is being examined experimentally.

APPROACH: N-propyl-N'-nitro-N-nitroso-guanidine (PNNG), a weaker stomach carcinogen than MNNG, is administered orally to rats and induction of intestinal metaplasia and stomach cancer is examined.

PROGRESS: (1) Administration of 59.5 micrograms/ml PNNG for 12 months induced intestinal metaplasia in glandular stomach in 80 percent of the rats 15 months later (Z. Krebsforsch., in press). (2) Administration of N-alkyl-NNG's with alkyl chains of four or more carbon atoms to rats as 0.34 mM solution for 12 months induced intestinal metaplasia at a low incidence but no gastric carcinoma was induced (GANN, in press). (3) Sequential observation of induction of intestinal metaplasia and gastric carcinoma in rats by PNNG revealed that the appearance of intestinal metaplasia (19 weeks) preceded that of gastric carcinoma (69 weeks).

49. STUDIES OF EXPERIMENTAL STOMACH CANCER

Matsukura, N., Kawachi, T., Sugimura, T., Ministry of Health & Welfare, Biochemistry Div., *5-1-1 Tsukiji, Chuo-ku, Tokyo, Tokyo, Japan*

OBJECTIVE: Promotion or inhibition of production of experimental stomach cancer by various agents.

APPROACH: The promoting effect of croton oil on gastrocarcinogenesis by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) is examined in rats.

PROGRESS: Gastric carcinomas were found in rats given MNNG for 3 months, followed by croton oil. No gastric carcinomas were found in rats given only MNNG for 3 months (Z. Krebsforsch., in press).

50. EXPERIMENTAL TREATMENT BY LOCAL IRRADIATION AND CHEMOTHERAPY OF MNNG OR ENNG INDUCED CANINE STOMACH CANCER

Koyama, Y., Maruyama, K., Kitagawa, T., Kakei, M., Omori, K., Ministry of Health & Welfare, National Cancer Center, *5-1-1 Tsukiji, Chuo-ku, Tokyo 104, Tokyo, Japan*

OBJECTIVE: The new methods of non-surgical treatment for gastric cancer.

APPROACH: MNNG or ENNG induced canine stomach cancers are used. Non-surgical treatment consists of local irradiation and chemotherapy.

PROGRESS: Endoscopic implantations of various small sources in the gastric lesions will be attempted. Work on an improved model of the endoscopic forceps is in progress.

51. ZINC, NITROSAMINE AND ESOPHAGEAL CANCER

Newberne, P. M., Massachusetts Inst. of Technology, School of Science, Dept. of Nutrition & Food Science, *77 Massachusetts Ave., Cambridge, Massachusetts,* 02139, United States of America

This study will determine the effects of a deficiency and excess of zinc and an excess of vitamin A on development of esophageal tumors in rats given methylbenzyl nitrosamine. Since alcohol consumption is associated with cancer of the esophagus in U.S. males this factor will also be examined. Preliminary studies have shown a derangement in tissue concentration of zinc and other trace elements and a significantly shorter lag period in tumor development suggesting a potential risk in marginal zinc deficiency. An excess of zinc will be given rats to determine effects on tumor inhibition. Two zinc dependent enzymes will be determined. Tissue distribution of labeled nitrosamine will be evaluated in deficient and control animals.

52. INDUCTION OF COLON TUMORS IN GUINEA PIGS

Cockerell, G. L., Cornell University, Ithaca Campus, New York State College of Veterinary Medicine, Dept. of Pathology, *825 Veterinary Research Tower, Ithaca, New York,* 14853, United States of America

OBJECTIVE: Develop an animal model of large bowel cancer as it occurs in humans; compare immunotherapeutic and surgical approaches to the treatment of large bowel cancer; study methods of early detection of tumors and define the gross microscopic staging of tumors progression or regression before and after treatment.

APPROACH: Animal model. Adenocarcinoma of the colon in the guinea pig will be induced by the intrarectal instillation of the chemical carcinogen methylnitrosourea (MNU). Immunotherapy vs. surgery: Guinea pigs will either receive a direct intratumoral injection of a cell wall preparation of mycobacterium bovis strain BCG or undergo surgical excision of the primary tumor. Controls will receive no tumor treatment. Comparisons between treatments will be made for time to death and behavior of primary and secondary tumors. Early diagnosis, tumor staging: Sequential studies will be be performed during tumor induction and following treatment, including analysis for fecal blood, colonoscopic examination and gross and histological examination of tumor sites.

B. PANCREATIC AND LIVER CANCERS INDUCED BY NITROSO COMPOUNDS

53. THE PATHOGENESIS OF CANCER INDUCED BY CHEMICALS

Farber, E., University of Toronto, Banting Inst., Dept. of Pathology, *100 College St., Toronto, Ontario,* M5G 1L5, Canada

OBJECTIVE: The objective is to develop a model that will allow the sequential analysis of the steps in the development of cancer between the initial exposure to a chemical carcinogen and the appearance of malignant neoplasia. The long-range objective of this study is to develop new approaches to the early diagnosis and therapy of pre-cancerous conditions and to generate new insights into the essential nature of neoplasia.

APPROACH: We are focussing our study on the rat liver since this organ probably requires a large number of steps and will allow the identification of early changes with a new approach. The approach is based on the development of a new assay for an early, if not the first, altered cell induced by a carcinogen and the subsequent control of this population through environmental modification.

PROGRESS: Progress has been made on several fronts in this research. The most striking relates to the analysis of initiation of liver carcinogenesis with chemicals such as diethylnitrosamine and methylnitrososourea. The initiation process consists of at least two-steps; the induction of a biochemical lesion, and its fixation by a cycle of cell replication. The replication can be a response to the induction of acute liver cell injury or to partial hepatectomy. We have now established in clear terms that liver cell necrosis can play a major role in initiating carcinogenesis. The possible importance of this in the genesis of liver cancer in man is intriguing.

54. SYRIAN HAMSTER MODEL OF PANCREATIC CARCINOGENESIS

Homburger, F., Adams, R. A., Bio-Science Lab., *7600 Tyrone Ave., Van Nuys, California,* 91405, United States of America

The objective of this proposal is to refine the Syrian hamster model of pancreatic cancer. Using N-nitrosobis (2-oxopropyl) amine (BOP), a compound which has been shown to induce high incidences of pancreatic ductal adenocarcinoma in randomly bred hamsters, we propose to screen our stocks of inbred hamsters in dose-response formats to determine which are the most susceptible to pancreatic tumor induction and to improve responsiveness (shorten latency) by hybridization of the most susceptible lines. We also propose to transplant BOP-induced pancreatic tumors to determine whether morphologic features are retained on serial transplantation in syngeneic hamsters to provide materials and background information relevant for chemotherapy studies for another proposal to be made at a later date.

55. MECHANISM OF CHEMICALLY INDUCED HEPATOMAS AND OVARIAN TUMORS

Thorgeirsson, S. S., Downs, R. W., Smith, C. L., Mattison, D. R., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Biochemical Pharmacology Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The long-range purpose of this project is to study the mechanism of experimentally induced hepatomas and ovarian tumors. We are particularly interested in the role of environmental contaminants such as the polycyclic hydrocarbons, nitroso compounds and N-acetylarylamines in initiating these tumors, as well as the role of genetic predisposition in development of these tumors. These tumors, at various time points during their development, will then be used as models for evaluating chemotherapeutic agents and other drugs which might be of therapeutic value. Topics of present interest are: (1) the role of metabolic activation, as well as the genetic control of activating enzymes, in the chemical initiation of hepatoma and ovarian tumors; (2) the time course of hepatoma and ovarian tumor initiation with particular emphasis on the changing enzyme patterns as the cells become increasingly dedifferentiated; (3) the role of nucleophilic agents such as N-acetylcysteine, L-cysteine and cysteamine in preventing the chemical initiation of tumors; (4) the characterization of cAMP and the beta-receptor involvement in the chemical induction of hepatomas and the possible use of beta-blockers and/or beta-agonists in preventing the occurence of these hepatomas.

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56. MECHANISM OF LIVER CARCINOGENESIS BY TWO NITROSOUREAS

Mirvish, S. S., Pour, P., Lawson, T., Ross, A., University of Nebraska, Medical Center, School of Medicine, Dept. of Biochemistry, *42nd & Dewey Aves., Omaha, Nebraska,* 68105, United States of America

We found that the nitrosoureas 1-nitroso-5,6-dihydrouracil (NDHU) and 2-hydroxyethylnitrosourea (HENU), administered chronically per os to rats, were strong carcinogens for the liver, and that HENU was also a strong carcinogen for the duodenum. Our main objective is to explain the biochemical and biological mechanisms by which these two compounds act in the liver and duodenum. Accordingly, we shall investigate whether NDHU and HENU alkylate liver nucleic acids (especially DNA) at different sites from other nitrosoureas which are not liver carcinogens, or cause alkylation that persists longer; cause breaks in the liver DNA molecule as observed by sucrose gradient sedimentation; have specific inhibitory and/or enhancing effects on liver DNA synthesis; are concentrated in the liver or metabolized there to proximate carcinogens; and induce hyperplastic liver nodules when combined with certain other treatments. We shall also explore similar parameters which may explain why HENU acts in the duodenum. As negative controls, parallel studies will be undertaken with ethylnitrosourea and l-nitrosohydantoin.

57. MEMBRANE GLYCOPROTEINS DURING HEPATOCARCINOGENESIS

Walborg, E. F., Hixson, D. C., Allison, J. P., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., *c/o Texas Medical Ctr., Houston, Texas,* 77030, United States of America

Chemically induced hepatocarcinogenesis is accompanied by the emergence of new cell populations exhibiting defective control of cellular proliferation and positional order, a phenotypic expression that presumably involves qualitative or quantitative alterations of cell-surface macromolecules, including the plasma membrane glycoproteins. The objective of this research is to delineate the molecular basis of qualitative differences in the plasma membrane glycoproteins of normal and malignant rat liver cells, focusing on alterations in their composition, structure, topography and dynamics. Cell populations to be studied include hepatocytes, primary and transplantable hepatocellular carcinomas and focal, proliferative lesions associated with hepatocarcinogenesis. Hepatocarcinogenesis in the ACI rat, induced by 2-acetylaminofluorene and diethylnitrosamine, will be used as the experimental model. Cells will be radiolabeled using methods specific for cell-surface glycoconjugates, e.g., oxidation with NaIO4 or D-Gal oxidase followed by reduction with NaB3H4, procedures that label sialic acids and D-Gal/D-GalNAc residues, respectively. Radiolabeled glycoproteins will be solubilized with nonionic detergent (Nonidet P-40) and resolved by polyacrylamide gel electrophoresis and affinity chromatography using carrier bound lectins. The major plasma membrane glycoproteins will be characterized with regard to their extractability from the membrane, liability to protease, primary structure as determined by mapping of tryptic peptides, and immunological cross-reactivity. Immunocytochemical techniques, using monoclonal antibodies prepared against selected glycoproteins, will be employed to assess the topographic and dynamic aspects of the integration of glycoproteins into the plasma membrane. Qualitative alterations of the plasma membrane glycoproteins will be correlated with the carcinogenic process, malignant transformation and selective processes involved in tumor progression.

C. BLADDER AND RENAL TUMORS INDUCED BY NITROSO COMPOUNDS

58. MULTISTAGE CARCINOGENESIS IN THE RAT URINARY BLADDER - FURTHER INVESTIGATION OF THE PROMOTION PHASE

Hicks, R. M., University of London, Middlesex Hospital Medical School, School of Pathology, Dept. of Cell Pathology, *Riding House St., London W1P 7LD, England, United Kingdom*

OBJECTIVE: To investigate the ability of alkylating agents and therapeutic drugs such as methylmethane sulphonate, cyclophosphamide, thiotepa and epodyl to promote tumor growth in an initiated urothelium.

APPROACH: Rats are given an initiating (sub-carcinogenic) dose of the carcinogen methylnitrosourea by a single intravesicular instillation into the urinary bladder. They are then subjected to prolonged treatment with the alkylating agent under investigation for promoting activity. Appropriate controls are established which receive either the initiating carcinogen alone or the suspect promoting agent alone. Tumor incidence is recorded after maintaining the animals for 2 years. The histology and ultrastructure of the urothelium are studied and the results are compared to assess pathological change attributable to each drug per se, and in combination with the initiator.

PROGRESS: Some experiments are completed but the histopathology has still to be assessed. Work with methylmethane sulphonate has already been started and the animals will be maintained for another 20 months before killing.

59. FURTHER INVESTIGATIONS WITH A 2-STAGE MODEL DESIGNED TO DETECT LOW POTENCY BLADDER CARCINOGENS AND CO-CARCINOGENS

Hicks, R. M., Severs, N., Chowaniec, J., University of London, Middlesex Hospital Medical School, Dept. of Cell Pathology, *Riding House St., London W1P 7LD, England, United Kingdom*

OBJECTIVE: To further develop our rabbit bladder model for detecting weak bladder carcinogens and co-carcinogens and to investigate the biological prinicples underlying multistage carcinogenesis, initiation and promotion.

APPROACH: We have developed a rat model which employs the principle of 2-stage carcinogenesis, to test compounds with suspected carcinogenic or co-carcinogenic potential for the bladder. The urothelium is exposed to a low dose of a known bladder carcinogen, and the test compound then administered to the animal by an appropriate route. If the test compound is also organotropic for the bladder and is either a solitary carcinogen, co-carcinogen or a promoter, it will increase the yield of tumors obtained with the low dose of the initiating carcinogen on its own. The model is also being used to investigate the dose response of the urothelium to a) the initiator and b) some of the test compounds; to determine the duration of the initiating effect; to compare the effect of single and 'split' doses of the same carcinogen; to identify useful and reliable initiators of carcinogenesis in the urothelium; and to study in depth the subcellular changes associated with the early, clinically 'silent' stages of neoplastic growth in the urothelium.

PROGRESS: N-methyl-N-nitrosourea (MNU) was used as the initiating carcinogen for a series of experiments. Saccharin and cyclamate both greatly increase the yield of bladder cancers in the MNU-initiated bladder and we conclude these compounds are weak initiators but powerful promoters of carcinogenesis in the rat bladder. 2-naphthylamine was both synergistic with MNU and also a solitary carcinogen producing an incidence of approximately 25 percent bladder cancer when tested at high doses on its own. Coffee and caffeine did not significantly increase the tumor yield after initiation with MNU.

60. IN VIVO BLADDER CARCINOGENESIS OF NITROSOAMINES

Oyasu, R., Miyata, Y., Izumi, K., Northwestern University, Chicago Campus, School of Medicine, Dept. of Pathology, *303 E. Chicago Ave., Chicago, Illinois*, 60611, United States of America

The immediate objective of the proposed investigation is to ascertain whether urine contains a factor(s) which serves as a promoting agent(s) in urinary bladder neoplasia. Our studies are based on the hypothesis that urine may contain minute amounts of carcinogen(s) derived from either exogenous or endogenous sources and that these induce cancer only when they are present in sufficiently high concentration or act in concert with a second substance in urine which may serve as a co-carcinogen or as a promoter.

Available evidence indicates that the urine contains a number of substances which are known to stimulate cell growth, and it is conceivable that some of them may play the role of either tumor promoter or co-carcinogen. The proposed investigation is intended 1) to study the role of urine, specifically its possible role as a co-carcinogen and/or a promoter - as a study model, heterotopically transplanted bladders (HTB) of rats diverted from urine flow will be used; 2) to study the role of urinary constituents as possible co-carcinogens and/or promoters - the substances to be tested will be epidermal growth factor and polyamines; and 3) to test suspected proximate carcinogens using HTBs as a model.

61. EXPERIMENTAL PATHOLOGY OF RENAL CARCINOGENESIS

Hard, G. C., Magee, P. N., Temple University, School of Medicine, Dept. of Pathology/Anatomy, *3322 Broad St., Philadelphia, Pennsylvania*, 19122, United States of America

The objective of the proposed research is to identify the sequential stages of tissue and cellular events which occur during the induction of renal neoplasia by nitroso compounds and to develop further cell culture models which will provide information that will correlate directly with the in vivo process. Emphasis will be placed on the identification of target cells and their responses to carcinogens, and on the identification of precursor lesions and their progression, utilizing techniques of isotopic labeling and electron microscopy. Cell cultures derived from carcinogen-treated animals will provide simpler experimental systems facilitating the analysis of target cells in terms of their ultrastructural, proliferative, biochemical and functional characteristics and of the stages in the transformation process. Additionally, it is proposed to seek and test for modifying factors effective in the tumor systems and to investigate their mode of action in the pre-determined stages of renal tumor induction. The broad objectives of the project are to further the understanding of the basic biology of human renal neoplasia and of the process of chemical carcinogenesis in general, and to identify strategies which may reverse or inhibit the cancer process.

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62. BIOCHEMICAL MECHANISMS IN EXPERIMENTAL BLADDER CANCER

Irving, C. C., Daniel, D. S., Wright, R. K., Rozzell, J. A., Murphy, W. M., University of Tennessee, Center for the Health Sciences, School of Medicine, Dept. of Urology, *62 S. Dunlap St., Memphis, Tennessee*, 38103, United States of America

The main objective of these studies is to learn more about the mechanisms by which known bladder carcinogens induce urinary bladder cancer in experimental animals. The model system that has been selected for study in this project is the induction of bladder cancer in rats with N-butyl-N-(4-hydroxybutyl)nitrosamine (BHBN) and its principal urinary metabolite, N-butyl-N-(3-carboxypropyl) nitrosamine (BCPN). The urinary bladder is the only organ in the rat in which cancer is induced by the administration of BHBN or BCPN, and the incidence of bladder cancer in exposed rats is 100%. More than 90% of these bladder cancers are transitional cell carcinomas.

The research proposed in this application is directed towards learning what metabolite of BHBN or BCPN is involved in the induction of bladder cancer, i.e., how are these nitrosamines metabolically activated? The hypothesis being tested is that a stable precursor of an activated metabolite derived from BCPN is formed in the liver and is excreted in urine, where it undergoes hydrolysis (either intravesically or intracellularly after absorption) with the liberation of an activated metabolite. At the present time, we do not believe that BCPN itself is metabolically activated directly by rat bladder epithelial cells.

63. IMMUNOTHERAPY OF BLADDER TUMORS IN A RAT MODEL SYSTEM

Harris, S. C., Paque, R. E., Lamm, D. L., Winters, W. D., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *San Antonio, Texas,* 78284, United States of America

The objective of this program is to establish and define a clinically relevant rat bladder tumor model for use in evaluating various immunotherapeutic and immunochemotherapeutic approaches to the treatment of carcinoma of the bladder.

Bladder tumors will be induced in Fischer 344 rats by oral administration of the chemical carcinogen, FANFT (N-(4-(5-nitro-2-furyl)-2-thiazolyl) formámide). The effects of such parameters as exposure interval and dietary lipid content will be investigated.

Bacillus Calmette-Guerin cell walls (BCG-CW) will be injected directly into the tumors to nonspecifically boost the host's immune respone to its tumor. The effect of the incorporation of mitomycin C, cis-platins or adriamycin into this protocol will also be investigated. Parameters of cellular and humoral immunity will be followed. These studies should be directly applicable to treatment of bladder cancer in humans.

Studies to date have established that 22 weeks of 0.2% FANFT will induce tumors in 100% of rats. Initial studies of the effects of intralesional injection of BCG-CW are currently being completed. However, it is clear that high dose BCG-CW combined with intravesical live BCG results in enhancement of tumor growth. An agarose microdroplet direct macrophage migration inhibition assay was developed as an in vitro assay of cellular immunity. An in vivo delayed cutaneous hypersensitivity assay using footpad and ear testing sites was established. A solid phage RIA for detecting circulating antibody to BCG antigens was developed. The excellent correlation between these cellular and humoral assays suggests that immunologic responsiveness in subjects receiving nonspecific immunostimulation with BCG or its fractions may be effectively monitored by simple periodic serologic assay for circulating antibody to BCG antigens.

D. BRAIN AND OTHER NERVOUS SYSTEM TUMORS INDUCED BY NITROSO COMPOUNDS

64. AN INVESTIGATION OF THE CARCINOGENIC ACTION OF NITROSAMIDES AND RELATED COMPOUNDS USING TISSUE CULTURE TECHNIQUES

Roscoe, J. P., Hince, T. A., Claisse, P. J., University of London, Middlesex Hospital Medical School, Dept. of Cell Pathology, *Riding House St., London W1P 7LD, England, United Kingdom*

OBJECTIVE: To identify changes in cell properties which occur during nitrosamide-induced carcinogenesis in the rat brain and to investigate the relationship of these to malignant transformation.

APPROACH: Pregnant rats (inbred BD IX line) are injected with ethylnitrosourea (ENU) on the 15th or 16th day of pregnancy. This transplacental exposure results in the induction of cerebral gliomas in a large proportion of the offspring. Cultures are prepared from the brains of foetuses or cerebra of offspring at a number of times after exposure to ENU but before the appearance of a visible tumor. Control cultures are prepared from animals exposed transplacentally to buffer. The cultures are examined for several features found characteristic of cells cultured from gross gliomas, e.g., tumorigenicity in newborn syngeneic animals, specific cellular morphology (phase contrast microscopy), growth in agar, fibrinolytic activity, altered surface morphology (scanning electron microscopy). Particular attention is being paid to the possibility that these characteristics are acquired at different times following exposure to ENU.

PROGRESS: The sequential in vivo-in vitro approach has shown that the presence of malignant cells can readily be demonstrated about half-way through the latent period. However, potentially malignant cells which only become tumorigenic after many months in culture are already present two days after exposure to ENU. These results are consistent with a multistage process of carcinogenesis. Further results suggest that fibrinolytic activity precedes the ability to form colonies in agar. A more detailed investigation of the sequence of changes and their relationship to each other is now being undertaken.

65. NERVOUS SYSTEM-SPECIFIC CHEMICAL CARCINOGENESIS

Rajewsky, M. F., Kirstein, U., Ahrens, O., Nehls, P., Muller, R., University of Essen, Inst. fur Zellbiologie, *Hufelandstrasse 55, D4301 Essen, Nordrhein West., Federal Republic of Germany*

Oncogenesis in pre- and postnatal rat brain by the direct-acting pulse-carcinogen N-ethyl-N-nitrosourea (EtNU) provides a model system for the study of chemical carcinogenesis. A single pulse of EtNU specifically results in a high incidence of neuroectodermal neoplasms; the magnitude of the carcinogenic effect is a function of the developmental stage of the nervous system during which the EtNU-pulse is applied (high-carcinogenicity after exposure during pre- and early postnatal age; decreasing carcinogenicity with increasing age). Fetal rat brain cells transferred to cell culture after exposure to EtNU in vivo undergo malignant transformation after a sequence of phenotypic alterations (including e.g., reduced substrate adherence, morphological transformation, soft agar colony formation, increased growth rate, increased plasminogen activator activity). We intend to compare the probability of neoplastic transformation between different tissues and between phenotypically differing cell populations within a given tissue (brain), as a function of their proliferative and differentiated (developmental) state at the time of exposure to EtNU. In particular we intend to investigate whether the cellular capacity to recognize, eliminate, and repair carcinogen (EtNU)-modified DNA components is connected with the probability of neoplastic transformation; and whether enzymic excision capacity for defined, potentially mutagenic DNA lesions (such as O6-ethyldeoxyguanosine (O6-EtdGuo) varies according to the type and differentiated (developmental) stage of the target cells. These studies are, therefore, concerned with the enzymology of excision repair of ethylated bases in DNA (chromatin), and will compare, with the use of newly developed, highly sensitive immunological detection methods for O6-EtdGuo, the elimination rates of O6-EtdGuo from DNA as well as structurally different fractions of chromatin, in different rat tissues, neural and non-neural cell types, and during different stages of prenatal and postnatal development.

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66. EARLY CHANGES IN POTENTIAL BRAIN TUMOR TISSUE

Soukup, S. W., Childrens Hospital Medical Center, *3245 Burnet Ave., Cincinnati, Ohio,* 45229, United States of America

Ethylnitrosourea (ENU) is a powerful transplacental carcinogen. A single dose to a pregnant rat produces neural tumors in adult offspring many months later. The long latency period offers an opportunity to look for early oncogenic changes in target neural tissues, applying a combination of in vivo and in vitro techniques.

We have demonstrated that tumorigenic doses of ENU produce chromosome aberrations in bone marrow and fetal tissues of treated animals as well as in cultured cells in vitro, suggesting that a mutation could be an indication, and possibly an explanation, for the initial oncogenic effect.

Rat neural tumors produced by ENU have been grown in cell culture. In cell lines from the gliomas and Schwannomas we have compared chromosome constitution, growth characteristics in culture, histological findings relating to the tumor and tumorigenicity of the cells. In 12 tumor lines excess chromosome 4 was present as a common chromosome finding, suggesting that extra chromosome 4 can be useful as a marker for ENU neural tumors.

Our main objective is to study early stages of oncogenesis in neural target tissues. Brain and trigeminal nerve ganglia are removed from rats treated transplacentally with ENU. At various stages postnatally neural tissues are initiated in cell culture and monitored for oncogenic transformation, cytogenetic changes and tumorigenicity.

We will attempt to determine whether excess chromosome 4 is related to ENU as a specific etiologic agent by studying the chromosome constitution of non-neural ENU tumors, or whether the chromosome is associated with a particular tumor cell

type, by studying rat brain tumors induced by other agents. The role of chromosome 4 in initiation or progression of the tumors will also be investigated by examining direct preparations of tumors with special emphasis on a search for competing cell lines and subclones.

67. NEURO-ONCOGENESIS BY RESORPTIVE CARCINOGENS

Koestner, A., Ohio State University, Columbus Campus, School of Veterinary Medicine, Dept. of Veterinary Pathology, *1900 Coffey Rd., Columbus, Ohio,* 43210, United States of America

Etiology and pathogenesis of human brain tumors are unknown. The high incidence of brain tumors in rats following single or multiple extraneural applications of N-nitroso compounds suggests that brain tumors in man may be induced by a single pre- or postnatal contact with extrinsic or intrinsic neuro-oncogenic agents. Since N-nitroso compounds occur in nature and human contact with these substances is likely, it is proposed to use these specific neuro-oncogenic agents as a model for the investigation of the induction phase of brain neoplasms. The molecular-chemical interactions of these compounds and their catabolites with cellular components will be investigated. The model will be used to study immune mechanisms of brain tumors with the ultimate goal of developing a unique approach toward immune therapy. It will also be investigated whether the direct interactions of these compounds with the cellular genetic apparatus are solely responsible for neoplastic transformation or whether the effect of these compounds is a result of activation of an endogenous tumor virus.

68. STUDIES IN NEURO-ONCOGENESIS AND NEUROGENESIS

Zagon, I. S., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Anatomy, *500 University Dr., Hershey, Pennsylvania,* 17033, United States of America

The proposed investigation is designed to elucidate the effects of transplacental administration of N-ethyl-N-nitrosourea (ENU) on the postnatal development of rodent cerebellum and hippocampus. Since ENU-treated animals may develop tumors of the cerebellum and hippocampus, and tumorigenic incidence is highest during morphogenesis, this proposal hypothesizes that detectable developmental errors may be produced that might result in a predisposition to neuro-oncogenesis. A major emphasis will be placed on morphology, and several research methods (histology, histochemistry, electron microscopy) will be utilized; whenever possible, quantitative and stereologic analyses will be included. Autoradiographic studies of cell proliferation and differentiation, as well as the localization and fate of 14C-ENU, will also be conducted and correlated with structural observations. This research will delineate the teratogenic action of ENU, define the role of neurogenesis during chemical carcinogenesis, and explore the etiology and pathogenesis of brain tumors.

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69. ETHYLNITROSOUREA-INDUCED RAT GLIOMAS

Spence, A. M., Hellstrom, K. E., Hellstrom, I. E., University of Washington, School of Medicine, Dept. of Medicine, *A-300 Health Sciences Ctr., SC-64, Seattle, Washington,* 98195, United States of America

The aim of this research is to extend our understanding of host responses to ethylnitrosourea (ENU)-induced F-344 rat malignant gliomas in order to elucidate the role of immunity in the pathogenesis of human glial neoplasms and to explore the feasibility of immunotherapy for these tumors. Following transplacental induction of malignant astrocytic and mixed gliomas with ENU, transplantable derivatives of several of these autochthonous tumors have already been produced by direct animal and alternate culture to animal serial passage methods. This research deals with four early-vintage transplantable glioma lines, two of which are immunogenic and two which elicit tumor-stimulatory responses in immunized hosts. In vivo tumor rejection assays and Winn tumor neutralization tests are employed: (a) to determine if these gliomas express individually unique or cross-reactive tumor specific transplantation antigens and (b) to measure the extent to which rejection and stimulation responses are active when these tumors are grafted in the brain. BCG-priming/PPD-coupling combine non-specific and specific methods of immunizing animals against tumor grafts. Experiments are added to assess the potential of these methods in augmenting immunologically mediated rejection or enhancement of gliomas implanted at either subcutaneous or intracerebral sites. Because the role of thymus-derived cells in mediating tumoricidal anti-glioma immunity or glioma growth-stimulatory processes has not been documented in vivo, investigations are planned to clarify T-effector and T-suppressor cell function in glioma immunity. Specifically, these studies include: first, tumor rejection assay in hosts rendered T-cell depleted by thymectomy; second, similar assays in chimeric animals reconstituted with thymus cells from tumor-bearing donors after treatment consisting of thymectomy/splenectomy/total-body irradiation; and third, Winn tests in which lymphoid elements, donated by glioma bearing hosts, are combined with glioma cells in vitro and then implanted in previously prepared glioma-immunized hosts.

70. IMMUNITY AGAINST NITROSOUREA-INDUCED RAT BRAIN TUMORS

Spence, A. M., University of Washington, School of Medicine, Dept. of Medicine, *A-300 Health Sciences Ctr., SC-64, Seattle, Washington,* 98195, United States of America

One hundred and eighty-seven glial malignancies have been generated in 128 offspring of 14 pregnant F-344 rats treated transplacentally with the resorptive, neurotropic carcinogen, ethylnitrosourea (ENU). In order to generate glioma lines for immunological experiments, 83 of such gliomas have been processed for (a) tumor cell cultures and (b) direct transplantation passages or alternate culture-to-animal passages to subcutaneous and intracerebral sites of syngeneic, sex-matched recipients.

Direct transplantation of ENU-induced gliomas to the intracerebral site of new hosts has yielded successful growths which have maintained the morphological features of parent glial tumors. Passage of ENU-induced gliomas to the subcutaneous site of new hosts either directly or by means of alternate culture-to-animal passage has yielded growths that, in a high proportion of cases, contain sarcomatous elements.

Immune reactions against ENU-induced gliomas in vivo and in vitro are being investigated and experiments are being designed to determine the source of sarcomatous elements in subcutaneous glioma transplants.

E. LUNG CANCERS INDUCED BY NITROSO COMPOUNDS

71. CO-CARCINOGENICITY OF ENVIRONMENTAL METALS AND MINERALS WITH SYSTEMIC CARCINOGENS IN THE RAT LUNG (ABBREV)

Heath, J. C., Strangeways Research Lab., Dept. of Cancer Research, *Wort's Cswy., Cambridge, England, United Kingdom*

OBJECTIVE: To investigate the carcinogenic action of very small lung deposits of mixed particulate minerals and metals of environmental importance, acting alone or together with a very low level of a systemic carcinogen.

APPROACH: The particulate minerals and metals are administered in a small amount by a single intratracheal instillation under anaesthesia. After about 6 weeks, when the pulmonary lesion is well established, a short course of once-weekly subcutaneous injections of a very low dose of a systemic carcinogen such as a nitrosamine is given. No further treatment is given, and the animals are allowed to live out their lives or to live until increasing respiratory distress renders their destruction desirable. The lungs and other relevant internal organs of all rats are fully examined postmortem.

PROGRESS: Lung lesions induced by minimal deposits of particulate silica or fibrous asbestos only very occasionally proceed to malignant metastasizing lung tumors. With the concurrent action of particulate cobalt, nickel or cadmium metals at 10% w/w of the total lung particulate deposit, rather more malignant lung tumors are produced. The further administration of a very low level of a systemic carcinogen such as a nitrosamine, acting systemically but at a level low enough not to

induce a significant number of malignant lung tumors by itself, can increase significantly the incidence of such tumors.

72. SUPPLY OF HAMSTERS TREATED WITH RESPIRATORY CARCINOGENS

Baxter, D. W., IIT Research Inst., *10 W. 35th St., Chicago, Illinois*, 60616, United States of America

Provide a steady weekly supply of a minimum of 100 Syrian golden hamsters which have been treated with a carcinogenic regimen of benzo(a)pyrene-ferric oxide and/or N-nitrosomethylurea.

73. STUDY OF RESPIRATORY CARCINOGENESIS

Little, J. B., Harvard University, Boston Campus, School of Public Health, Dept. of Physiology, *55 Shattuck St., Boston, Massachusetts*, 02115, United States of America

The Contractor shall: Determine the role of hematite carrier particles in radiation carcinogenesis; identify doses of BP and 210Po which, given individually, will result in a small but measurable tumor incidence; administer both BP and 210Po simultaneously and sequentially to determine synergistic action; and administer DEN systemically following treatment with either BP or 210Po to determine possible synergism of local and systemic carcinogens.

F. TRANSPLACENTAL CARCINOGENESIS BY NITROSO COMPOUNDS

74. TRANSPLACENTAL EFFECTS OF NITROSO COMPOUNDS

Meier, H., Fox, R. R., Bedigian, H. G., Jackson Lab., Virus-Leukemia Laboratory, *Box 258, Bar Harbor, Maine*, 04609, United States of America

We are analyzing the chronic transplacental carcinogenic effects of nitroso compounds in inbred strains of mice and rabbits. These effects depend greatly upon the hereditarily determined differences in susceptibility or resistance of pregnant mothers and embryos, and the stage of fetal (organ) development. Examples are the transplacental induction of primay renal tumors in rabbits, the transplacental effects of diethylnitrosamine in mice as related to strain of mice (genotype) and day(s) of administration during pregnancy, etc. We are also interested in the effects of chemical and viral (type C genomes) interaction in the fetuses.

75. IMMUNOBIOLOGY OF TRANSPLACENTAL CARCINOGENESIS

Bennett, M., Elkort, R. J., Boston University, School of Medicine, Dept. of Pathology, *80 E. Concord St., Boston, Massachusetts*, 02215, United States of America

The objective is to determine the role of immunocompetent cell dysfunction or lack of maturation that is associated with the high efficiency of transplacental carcinogenesis. We have observed that pregnant, but not other, Erythrocebus patas monkeys are immunosuppressed by the carcinogen, 1-ethyl-1-nitrosourea (ENU). Mice are resistant to ENU unless they are also injected with the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA). We will determine if ENU synergizes with TPA and/or pregnant monkey serum to immunosuppress mouse lymphocytes in vitro and in vivo. Vitamin A acid (retinoic acid) abrogates the promoting effect of TPA and prevents induction of ornithine decarboxylase (OCD). OCD produces putrescine. We will test the effect of retinoic acid on the immunosuppressive effects of ENU with TPA and on transplacental carcinogenesis itself.

76. CHEMICAL FACTORS IN FETAL ONCOGENESIS

Anderson, L. M., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

In a recent study we found that 10 ppb dimethylnitrosamine given in the drinking water of strain A mice from preconception until 22 weeks of age caused a significant increase in lung tumors. We are repeating and extending this experiment to include extended periods of time, endpoints up to 28 months, so that effects of longer term exposure to this amount of DMN can be determined.

In a parallel investigation, we observed that treatment of pregnant mice with poly-chlorinated biphenyls resulted in a large increase in dimethylnitrosamine demethylase activity in the livers of their progeny before weaning. The effect was mediated largely by lactational delivery of the PCBs. In a tumorigenicity assay in progress, we are testing the effect of maternal treatment with PCBs on the carcinogenicity of DMN given to the offspring between birth and weaning. We also intend to measure DMN metabolism in other organs of the young mice, especially lung, pancreas, and kidney.

Another possible environmental agent, N6(methylnitroso)adenosine, has been found to be a multipotential carcinogen in the mouse. We are studying the mechanism of its metabolic activation by high pressure liquid chromatography by determining and quantifying the types of products formed by different organ and subcellular preparations, the effect of inhibitors, etc. DNA modification experiments will also be attempted.

VI. EFFECTS OF RADIATION, DRUGS, DIET, AND OTHER FACTORS ON CARCINOGENESIS BY NITROSO COMPOUNDS

77. EFFECT OF X-IRRADIATION ON THE NEUROCARCINOGENIC EFFECT OF ETHYL-NITROSOUREA (ENU)

Knowles, J. F., Medical Research Council, Radiobiology Unit, *Harwell, Didcot, Oxfordshire OX11 ORQ, England, United Kingdom*

OBJECTIVE: To investigate whether X-irradiation has a synergistic or an inhibitory effect on tumors of the nervous system produced by ENU.

APPROACH: Newborn rats are injected with ENU during the first 24 hours of life and during the second 24 hours of life are irradiated.

78. ENHANCEMENT OF NBU-LEUKEMOGENESIS IN MICE BY INJECTION OF THYMUS CELLS

Shisa, H., Saitama Cancer Center, Dept. of Pathology, *818 Komoro, Ina-Machi, Kitaadachi-Gun, Saitama-Ken 362, Japan*

OBJECTIVE: To study the effect of thymus cells in N-nitrosobutylurea(NBU) leukemogenesis in a mouse system

APPROACH: C57BL/6J, CBA/H-T6(T6), AKR/J and its syngeneic substrain AKR/T1ALD(T1) mice were used. T6 and T1 mice have marker chromosomes. 1) Thymectomized (C57BL X T6)F1 mice were treated with NBU after either intraperitoneal injection of parental C57BL thymus cells or intraperitoneal insertion of thymus tissues in a diffusion chamber. 2) Sublethally irradiated AKR mice were treated with NBU after restoration by intravenous injection of (AKR X0T1)F1 spleen or bone marrow cells mixing with or without thymus cells of T1 mice. It is possible to determine the origin of the leukemic cells by marker chromosome. T-cell characteristics of leukemic cells were ascertained by the cytotoxicity test for theta-antigen.

PROGRESS: 1) An increased incidence of leukemia was observed in thymectomized mice only after thymus cells injection. The thymus in diffusion chamber has no effects on the development of leukemias. All the leukemias originated from host cells and have no theta-antigen on their cell surface. 2) A marked acceleration of leukemia development was noticed in irradiated mice which restored by cell injection mixing with thymus cells. In this particular system, leukemic cells originated from donor spleen or bone marrow cells, but not from injected thymus cells. Forty percent of thymus enlarged leukemias which restored by bone marrow cells were theta-negative whereas the majority of leukemias which occured in restored spleen mice or bone marrow with thymus cells restored mice were theta-positive. Further, our chromosomal analysis indicated that repopulation ability of spleen and bone marrow cells into the thymus can be accelarated by mixing with thymus cells in inocula.

These results suggest that thymus cells are not target cells of NBU and may somehow have influence on cell kinetics of lymphoid precursor cells in the spleen or bone marrow probably by cell to cell interaction.

79. COMBINED CARCINOGENIC EFFECT OF RADIATION AND CHEMICALS

Tanooka, H., Hoshino, H., Ministry of Health & Welfare, Radiobiology Div., *5-1-1 Tsukiji, Chuo-ku, Tokyo 104, Tokyo, Japan*

OBJECTIVE: To clarify the mechanism of additive carcinogenic effect of radiation and chemicals.

APPROACH: Target is the mouse skin. The skin was irradiated with low-penetrating beta rays in combination with painting with a chemical (e.g., 4-nitroquinoline 1-oxide, 4NQO). Skin tumor formation was then observed.

PROGRESS: With beta rays alone, the effect of dose-fractionation was found. The optimum condition for skin carcinogenesis is a repeated irradiation with 500-1000 rad. Single irradiation with 3000 rad alone did not produce a tumor. However, the latent effect persisted over one year and could be induced by combination with painting with 4NQO of sub-carcinogenic dose.

80. MODIFICATION OF CHEMICALLY INDUCED CANCERS IN ANIMALS WITH LONG-TERM EXPOSURE TO LOW LEVEL MICROWAVE FIELDS (ABBREV)

Szmigielski, S., Szudzinski, A., Pietraszek, A., Center for Radiobiology & Radioprotection, *128 Szaserow, 00909 Warsaw, Poland*

Rapidly growing environmental and occupational exposure to microwave radiation calls attention to the possible specific (non-thermal) biologic effect of this radiation, including delayed genetic phenomena and the possibility of neoplastic transformation of irradiated cells. Influence of long-term low-level microwave irradiation on cell mediated immune reactions is also postulated. These effects may differ, depending upon frequency of microwaves, time of exposure and power densities used. The following subjects will be elaborated in the project: 1. Development of skin cancer induced by 3,4-benzopyrene in mice exposed for 1-4 months to non-thermal (5 or 15 MW/cm2) power densities of 2450 MHz microwaves (continuous and pulse modulated waves); irradiation with microwaves will be performed prior to or simultaneously with treatment with 3,4-benzopyrene; cancer development will be evaluated macroscopically (7-range scale) and microscopically (routine histology and histochemistry). 2. Development of primary hepatomas in mice injected intraperitoneally with di-ethyl-nitroso-amine /DENA/ and exposed to 2450 MHz microwave field (as above). 3. Natural antineoplastic resistance (cancer lung colonies, natural killer activity) in mice exposed to low level microwave fields.

81. PROTEIN NUTRITION AND PRIMARY ACTION OF A CARCINOGEN

von der Decken, A., Eriksson, B., Wenner Gren Inst. for Experimental Biology, Dept. of Cell Physiology, *Norrtullsgatan 16, S11345 Stockholm, Sweden*

Nitroso compounds show great versatility as carcinogenic agents. Knowledge about dietary protection from the carcinogenic action of nitroso compounds is of prevailing interest in relation to carcinogenesis in humans. The aim of this investigation is to study the effect of administration of dimethylnitrosamine to mice fed a diet balanced in amino acids, or proteins enriched in sulfur-containing amino acids. The dietary conditions used will include high quality proteins at a 20% level and enrichment of the proteins with the sulfur-containing amino acids methionine and cysteine. These are introduced to test the theory that a reaction of the sulfur groups with dimethylnitrosamine intermediates will prevent interaction with the susceptible DNA molecules. The objectve is to investigate the protective influence that such a diet has on the occurence of single strand breaks in DNA caused by dimethylnitrosamine.

APPROACH: Quantitation of the breaks produced in DNA will be done by labeling with 125-iodine followed by separation of the denatured DNA in polyacrylamide gel electrophoresis and autoradiography. Labeling of DNA at the break points with gamma-32P-ATP and polynucleotid kinase (EC 2.7.1.78) will also be tested. DNA will be labeled at the free 5'OH group with 32P, and after separation by polyacrylamide gel electrophoresis radioactivity will be determined. A further step is formation of initiation complex for RNA synthesis with Escherichia coli RNA polymerase containing sigma factor (EC.2.7.7.6) and gamma-32P-ATP or gamma-32P-GTP as the initiation nucleotide. These may be replaced by 14C or 3H labeled ATP or GTP. The heparin resistant complex is precipitated, excess of radioactivity removed and the remaining radioactivity determined. A nonspecific initiation occurs in vitro at each single strand break.

82. DIET AND CHEMICAL CARCINOGENESIS

Wade, A. E., Bunce, O. R., Capomacchia, A. C., Holmes, D. T., Baker, M. T., University of Georgia, School of Pharmacy, Dept. of Pharmacology, *Athens, Georgia,* 30601, United States of America

The objectives of this research are to determine the effects of selected dietary states on the activity of the mixed function oxidases isolated from rat liver and lung which are responsible for the activation or inactivation of chemical carcinogens. Assessment of function will include measurements of (a) quantitative and qualitative changes in the metabolism of three model carcinogens, benzo(a)pyrene, 2-acetamidofluorene (2-AAF) and N-nitrosodimethylamine (DMN); (b) the influence of diet on the ability of the mixed function oxidases to alter the mutagenic potential of these carcinogens and (c) in vitro binding of these carcinogen metabolites to native calf-thymus DNA and other nucleoproteins.

83. ROLE OF SEX HORMONES IN HEPATOCARCINOGENESIS

Vesselinovitch, S. D., Goldfarb, S., Mihailovich, N., Rao, K. V., Pugh, T., University of Chicago, School of Medicine, Dept. of Radiology/Pathology, *5801 S. Ellis Ave., Chicago, Illinois,* 60637, United States of America

The overall objective of this project is to characterize the role of sex in the histogenesis of chemically induced hepatocelluiar carcinomas using mouse model developed at the University of Chicago. The latter consists of induction of hepatocellular carcinomas following single intraperitoneal injection of diethylnitrosamine (DEN) into C57BLxC3H F1 mice. Intact mice and mice gonadectomized at different intervals following carcinogenic treatment are utilized. At 3 days after injection of DEN, all experimental mice showed hepatic fatty changes with predominant central and mid-zonal distribution. However, these changes were not present any more in mice killed 7 days after DEN treatment. The earliest focal hepatocellular changes were identified at 10 weeks following DEN treatment. The lesions, referred to as basophilic hepatocellular foci (BHF) were small (six cells in diameter) or large (up to approximately 200 cells in cross-sectional area). This basophilia was due to the accumulation of RNA, since pretreatment of sections with RNAase completely abolished their staining with toluidine blue. The number and size of BHF increased with time. Higher dose of DEN (5.00 micrograms/g body weight) induced 5-fold greater number of BHF than the lower carcinogenic dose (1.25 micrograms/g body weight). Histochemical and autoradiography data are being analyzed.

84. SYNTHETIC STEROIDS AND HEPATOCARCINOGENESIS

Vesselinovitch, S. D., University of Chicago, School of Medicine, Dept. of Radiology, *5801 S. Ellis Ave., Chicago, Illinois,* 60637, United States of America

There is enough evidence that sex hormones may influence promoting phase of hepatocarcinogenesis apart from their effect upon initiation which operates through enzyme system activating chemical carcinogens. Clinical and experimental data show that males develop liver tumors more readily than females. Also, change in hormonal environment triggered by gonadectomy or administration of sex hormones following initiation modifies incidence of liver tumors, indicating strongly its modulating effect upon promotion of neoplastic process. Recent clinical observations suggest that use of oral contraceptives and anabolic steroids may be causally related to observed increase in the incidences of focal nodular lesions and even hepatocellular carcinomas in individuals using such hormones for long periods. The hypothesis that synthetic hormones may also achieve their effect by influencing promotion could be tested and defined in a system developed in our laboratory, in which single low dose levels of diethylnitrosamine (DEN) (less than or equal to 0.15 micrograms/g body weight) were shown to be effective in initiating hepatocarcinogensis in C57BLxC3H F1 mice. Slightly higher, but still low, dose levels (0.6 to 5.0 micrograms/g body weight) were able to induce nodular liver lesions, ranging from

hyperplastic to adenomatous and malignant cell populations, their incidence depending upon the dose of carcinogen used and the length of observation period. In the proposed study, promoting effect of norethynodrel, mestranol, methyltestosterone, and oxymetholone will be tested in groups of mice conditioned by subeffective (initiating) and marginally carcinogenic (inducing) single dose treatments with DEN by assessing type and incidence of developing lesions. Possible weak carcinogenic activity of the above hormones will be concurrently assessed. Phenobarbitone (0.05%) will be used as positive promoter.

85. INHIBITION OF BLADDER CARCINOGENESIS BY RETINOIC ACID

Squire, R. A., Loew, F. M., Strandberg, J. D., Arnold, E. A., Leary, S. L., Johns Hopkins University, School of Medicine, Dept. of Medicine, *725 N. Wolfe St., Baltimore, Maryland,* 21205, United States of America

A study of the potential inhibition of rat bladder carcinogenesis by 13-cis-retinoic acid is proposed. The carcinogen will be N-butyl-(4-hydroxybutyl) nitrosamine administered by gastric intubation twice per week for 6 weeks for a total dose of 1200 mg. The retinoid will be given continuously in the feed at 300 mg/kg of diet, beginning either prior to, simultaneously to or following carcinogen exposure. Possible inhibitory effects will be evaluated by sequential pathologic observation throughout the animal's life span and by evaluation of the levels of DNA, RNA and protein synthesis in the urotheljum. Specific attention will be given to cell proliferation, tumor incidence, progression or reversibility, histologic grading, invasion and metastases.

In order to assess the feasibility and safety of 13-cis-retinoic acid as an anti-neoplastic agent, subchronic toxicity will also be assessed in a 180 day feeding study at doses of 300, 600, and 900 mg/kg of diet. Complete clinical, pathological and biochemical values will be assessed as well as tissue bioaccumulation.

86. CHLORINATED WATER AND MEMBRANE FUNCTIONS AND NEOPLASIA

Sun, A. Y., Vogt, C. R., Sun, G. Y., Liao, J. C., University of Missouri, Columbia Campus, Sinclair Comparative Medicine Research Farm, *Rte. 3, Columbia, Missouri,* 65201, United States of America

Chlorine is suspected as a cancer risk because it interacts with organic matters in water and in biological systems leading to the formation of various toxic chloroderivatives. The overall objective of this proposed study is to investigate the interaction of chlorine and/or chlorinated compounds with the cellular constituents under the physiological conditions in relation to the biochemical mechanism of tumor formation as induced by this interaction. Specifically, we shall: (1) Study the effect of acute and chronic treatment of chlorinated water on the structure and function of biological membranes, (2) evaluate the synergism and promotion in tumorigenesis when chlorine water ingestion is combined with other chemical carcinogens, and (3) examine the inhibitory effect of dietary antioxidants on the chemical carcinogenesis as induced by chlorine and dimethylnitrosamine (DMN).

The interaction of chlorine with some major body constituents of rat, such as proteins, lipids, nucleic acids and nucleotides, at the cellular and subcellular level, will be investigated after administration of 36Cl-labeled hypochlorite by intragastric intubation. The change in the compositions (lipids and proteins) and functions (membrane-bound enzymes, transport activities) of erythrocyte membranes, kidney and liver mitochondria and synaptic plasma membranes will be studied and compared between control and experiment animals. The growth rate, tumor incidence and associated histological and membrane changes resulting from administration of chlorine either alone or combined with DMN will be examined. In addition, the inhibitory effect of dietary vitamin E and selenium on the chemical carcinogenesis as induced by chlorine alone or combined with DMN will be evaluated. It is anticipated that the results of this investigation will provide new information about the prevalence of mutagenic agents in drinking water and in the biological system. Possible preventive measures against the chemical carcinogenesis may be developed as a result of this study.

87. PREVENTION OF PANCREATIC CANCER IN ANIMALS BY RETINOIDS

Longnecker, D. S., Dartmouth College, School of Medicine, Dept. of Pathology, *P.O. Box 833, Hanover, New Hampshire,* 03755, United States of America

OBJECTIVE: To induce an incidence of carcinomas or preneoplastic epithelial lesions in pancreas of azerine-treated rats and N-nitrosobis(2-oxopropyl)amine-treated hamsters, and then to see if there is a decrease in the incidence, size, or number of such cancers or preneoplastic lesions in the rats and hamsters given retinoid-supplemented diets during the latent period of carcinogenesis.

88. DIETARY FIBER - THE PHYSICAL CHEMISTRY OF LIGNINS

Pethica, B. A., Zuman, P., Falkehag, I., Goring, D. A., Clarkson College of Technology, School of Arts & Sciences, Dept. of Chemistry, *51 Main St., Potsdam, New York,* 13676, United States of America

Certains forms of cancer are believed to be less common when the diet contains 'dietary fiber.' We propose to examine the physico-chemical properties of lignins in preparations partially or fully separated from plant fiber, and thereby to provide a basis for hypotheses concerning the role of this constituent in the diet in relation to carcinogenesis. The binding of several carcinogens and co-carcinogens to these lignins will be studied. The main emphasis will be on nitrosamines and other nitroso-compounds and bile salts. Lignin may also influence carcinogenesis through its oxidation-reduction properties, and through reaction with nitrates. It can also bind metal ions and other significant substances in the diet. The study will thus illustrate the effect of lignin on the bioavailability of a variety of substances implicated in carcinogenesis.

89. ISOPROTERENOL AND CARCINOGENESIS

Barka, T. D., City University of New York, Mount Sinai School of Medicine, School of Medicine, Dept. of Anatomy, *5th Ave. at E. 100th St., New York, New York,* 10029, United States of America

Isoproterenol (IPR), a beta-adrenergic drug, stimulates DNA synthesis in the parotid and submandibular glands. Repeated injections of this drug produce hyperplastic and hypertrophic enlargements of these organs. It is proposed to test the hypothesis that IPR may act as a tumor promoter in chemical carcinogenesis of the salivary glands. The possible tumor-promoting effect of IPR will be tested with two carcinogens, dimethylbenz(a)anthracene and methyl nitrosourea. The carcinogens will be given in low doses, followed by the chronic administration of IPR. Methyl nitrosourea will be given also at the peak of DNA synthesis induced by IPR, in order to test the significance of DNA synthesis in the carcinogenesis by this agent.

90. IN VIVO COCARCINOGENESIS OF CHEMICALS AND VIRUSES

Howett, M. K., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Microbiology, *500 University Dr., Hershey, Pennsylvania,* 17033, United States of America

The effect of coincidental exposure of animals to oncogenic viruses and chemical carcinogens will be studied. N-nitroso compounds (known carcinogens) will be administered to animals in conjunction with two DNA-containing tumor viruses: simian virus-40 (SV40) or herpes simplex virus type 1 (HSV-1). In one approach virus-specific tumors will be induced with SV40 and the effect of carcinogens on these tumors will be studied. In a second approach, HSV-1, a virus which has oncogenic potential but usually does not cause experimental tumors in vivo, will be given with suboptimal doses of N-nitroso compounds and possible synergism in the formation of tumors will be examined. Lastly, we will examine the role of virus infection on tumor induction by known chemical carcinogens. Evidence has already been obtained in this laboratory indicating that nontoxic doses of nitrosomethylurea can enhance herpesvirus specific transformation in vitro. It has been demonstrated that the maximum transformation rate occurs subsequent to the maximum alkylation of cell DNA. It is in light of this in vitro evidence, as well as work by others, that we have been encour-

aged to show in vivo cocarcinogenesis of chemical carcinogens with DNA tumor viruses.

91. BIOCHEMICAL MECHANISMS IN EXPERIMENTAL BLADDER CANCER

Irving, C. C., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Cancer Research Laboratories, *1030 Jefferson Ave., Memphis, Tennessee*, 38104, United States of America

The objective of this study was to determine if disulfiram (DSF; tetraethylthiuram disulfide; Antabuse) would influence the induction of bladder cancer in rats given N-butyl-N-(4-hydroxybutyl)-nitrosamine (BHBN). Adult male Wistar rats were divided into four groups as follows: (1) Control diet (Wayne Lab Blox); (2) control diet plus 0.025% BHBN in the drinking water; (3) control diet containing 0.5% DSF; and (4) control diet containing 0.5% DSF plus 0.025% BHBN in the drinking water. The animals were kept on these regimens for 15 weeks and then were transferred to and maintained on control diet. The average total intake of BHBN was 1.21 gm/rat for group 2 and 1.23 gm/rat for group 4. At the time the BHBN was discontinued (15 weeks), the urinary bladders of small numbers of animals in each group were examined and the incidence of bladder cancer was: group 1, 0/3; group 2, 3/9; group 3, 0/3; and group 4, 0/9. At 20-32 weeks (5-17 weeks after the BHBN was discontinued), the cumulative incidence of bladder cancer was: group 1, 0/6; group 2, 48/48 (100%); group 3, 0/6; and group 4, 1/30 (3%). Except for a carcinoma of the renal pelvis in one rat in group 2, no other tumors have been observed in any of these rats. Observation of the remaining rats in groups 1, 3, and 4 (approximately 20/group) continues. It is concluded that DSF has significantly inhibited (p less than 0.001) the induction of bladder cancer by BHBN. The mechanism of action of DSF in this process is under investigation.

VII. OTHER IN VIVO STUDIES OF CARCINOGENESIS BY NITROSO COMPOUNDS

92. THE BIOLOGY OF XENOPUS LAEVIS IN RELATION TO CARCINOGENESIS

Balls, M., Clothier, R. H., University of Nottingham, School of Medicine, Dept. of Human Morphology, *University Park, Nottingham NG7 2RD, England, United Kingdom*

OBJECTIVE: The initial aim is to follow up our finding that Xenopus laevis (the South African clawed toad) may be resistant to the carcinogenic effects of nitrosomethylurea, an alkylating agent, even though the DNA of liver and kidney cells is alkylated to the extent and in the positions within the DNA which are thought to be correlated with tumor formation in mammals.

The ultimate goal of the work is the production of cloned cultured cells of proven malignancy for investigations into the genetic basis of neoplastic transformation by nuclear transplantation.

APPROACH: In the new work, we plan to use a wider range of chemical carcinogens and treatment regimes than in our previous experiments.

93. INVESTIGATION OF THE SKIN CARCINOGENIC POTENCY OF DIFFERENT CHEMICALS

Iversen, O. H., Iversen, U., Kreyberg, L., Universitetet i Oslo, Inst. of Pathology, *Rikshospitalet, Oslo 1, Norway*

OBJECTIVE: To study the skin carcinogenic potency of different nitrosamines after topical application to the skin of hairless mice.

APPROACH: The skins of hairless mice are painted in single weekly paintings with N-nitrosomethylurea, dimethylnitrosamine, and diethylnitrosamine. The number of papillomas and carcinomas appearing is observed, and all the malignant tumors are histologically verified.

PROGRESS: It seems that N-nitrosomethylurea is a strong carcinogen, whereas the two other substances do not cause tumors on mouse skin.

94. CHEMICAL CARCINOGENESIS IN CYST INDUCED IN PRIMATES

Jonck, L. M., Raubenheimer, E. J., University of Pretoria, School of Dentistry, Institute of Stomatology, *P.O. Box 1266, Pretoria 0001, Transvaal, South Africa*

The carcinogenic action of nitro-amine derivates is well known. The aim of this project is to induce neoplastic change in an experimental cyst model developed after implantation of oral epithelium in the back muscles of immunosuppressed monkeys.

In this experiment particular attention will be paid to the concentration of the carcinogen and the time lapse to affect neoplastic change in oral epithelium. An attempt will also be made to study the influence of drugs used in dentistry known to be correlated with nitrosamines.

95. NORMAL AND NEOPLASTIC DEVELOPMENT OF THE MAMMARY GLAND

Dulbecco, R., Unger, M., Bologna, M., Bennett, D., Salk Inst. for Biological Studies, Dulbecco Laboratory, *10010 N. Torry Pines Blvd., P.O. Box 1809, San Diego, California*, 92112, United States of America

The studies on the epithelial cell types in the adult rat mammary gland and their evolution will be completed. The study of markers for various cell types will be continued; emphasis will be placed mostly on monoclonal antibody to various types of rat mammary cells. The types of cells involved in carcinogenesis by N-nitrosomethylurea will be defined on the basis of their developmental history and potential, and of available markers. The condition leading to dome formation in a spontaneously doming mammary cell line will be studied and possible effectors identified. The role of the Thy-1 antigen on dome formation will be investigated further.

96. MUTAGENICITY OF DIETHYLNITROSAMINE IN WASPS

Clark, A., Smucker, L., Hamilton, D., University of Delaware, School of Arts & Sciences, Dept. of Biology, *Newark, Delaware*, 19711, United States of America

Groups of 15 male parasitic wasps (Habro bracon) are being fed diethylnitrosamine in a diet of 10% sucrose solution, then mated with 15 virgin females for 12 hours in a dominant lethal study.

The numbers of eggs hatched, the stage of embryonic development at death, and the male/female ratio of hatched eggs will be studied as indicators of mutagenicity.

Groups of wasps will receive a 25 millimolar solution of ethylmethanesulfonate, a known mutagen, as positive controls. Negative controls will receive a 10% sucrose solution.

97. CARCINOGENESIS, CHEMOTHERAPY AND BIOLOGICAL MARKERS IN NON-HUMAN PRIMATES

Adamson, R. H., Sieber Fabro, S. M., McIntire, R. K., Correa, P., Dalgard, D. W., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Chemical Pharmacology Laboratory, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The carcinogenic effects of 26 substances, including antineoplastic and immunosuppressive agents, contaminants of human foodstuffs, rodent carcinogens, pesticides, and artificial sweeteners, are being evaluated in four species of non-human primates. Seventeen of these substances have not as yet demonstrated carcinogenic activity, although some have been undergoing tests for almost three years. Nine of the test compounds are carcinogenic in non-human primates, producing tumors in 9.1-100% of the treated animals. The effects of six of the nine compounds (DENA, DPNA, 1-nitrosopiperidine, aflatoxin B1, MAM-acetate and urethane) were manifested primarily as hepatocarcinogenicity. 1-methyl-1-nitrosourea induced squamous cell carcinomas of the oropharynx and esophagus, with the esophageal tumors possessing clinical and morphologic similarities to human esophageal carcinoma. Long-term treatment with procarbazine resulted in an increased incidence of malignancies, approximately one-half of which were AML. One of 10 monkeys treated with adriamycin also developed AML. Alpha-fetoprotein has been found to be a useful marker for diagnosing and for following the treatment of hepatocellular carcinoma in non-human primates. Studies on chemo-prevention of cancer using various nucleophiles are in progress.

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98. MAMMARY CARCINOMAS AND HEPATOMAS IN MICE TREATED WITH RETINYL ACETATE

Maiorana, A., Gullino, P. M., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Experimental studies have shown that retinyl acetate (a derivative of vitamin A) reduces the incidence of mammary carcinomas induced in rats by DMBA or NMU treatment. This work aims at establishing whether the same compound could prevent the appearance of mammary tumors in C3H-A mice, a strain in which genetic background and viral infection play a major role in the mammary tumorigenesis.

99. RECOGNITION OF VERY SMALL TUMORS

Sandor, T., Swensson, R. G., Rumbaugh, C. L., Schoene, W. C., Kido, D. K., Harvard University, Boston Campus, School of Medicine, Dept. of Radiology, *25 Shattuck St., Boston, Massachusetts,* 02115, United States of America

The proposed research would evaluate the utility of high-level radiographic magnification (up to 7X) for the early recognition of tumors. Both regular and tomographic magnification will be applied to in vitro and in vivo animal models that contain either tumors (V2 carcinomas and nitrosourea-induced tumors) or only normal vasculature. Perceptual studies and computer-aided densitometry will be used to determine how effectively magnification improves recognition of small tumors and blood vessels under specified imaging conditions. The resolution and noise properties of the employed film-screen combinations will be parameters. The goal is to develop quantitative relationships between these physical parameters and the perceptual and densitometric findings. The quality of perceptual performance will be determined by analysis of observers' ROC-curves for the in vitro and in vivo detection of tumors and for the detection and delineation of small blood vessels in digitally simulated imagery.

100. DIALLYLNITROSAMINE CARCINOGENESIS - SPECIES DIFFERENCES

Grandjean, C. J., Gold, B., Pour, P., Midwest Research Inst., *425 Volker Blvd., P.O. Box 27-303, Kansas City, Missouri,* 64180, United States of America

In general nitrosamines constitute a class of potent carcinogens that induce tumors in all rodent species, although the tumor sites do vary between species. There are only a few examples of non-carcinogenic nitrosamines that are not fully substituted at the alpha-positions and hence prevent alpha-hydroxylation (activation). Diallylnitrosamine (DAN) is one such compound and was reported non-carcinogenic in the BD rat. However, we have found DAN to be a potent inducer of respiratory tract tumors in the Syrian golden hamster upon multiple or a single subcutaneous (s.c.) injection. These comparative findings give rise to multiple questions concerning this type of species difference never observed before with nitrosamines.

To determine some of the factors that may contribute to this dramatic species difference in light of the present knowledge about nitrosamine carcinogenesis the following studies are proposed: (1) determination of the carcinogenic response of the Syrian golden hamster and the BD(IX) rat to DAN using intragastric and subcutaneous administration; (2) determination of whether DAN is metabolically converted into other nitrosamines in either species using both i.g. and s.c. routes of administration; (3) determination of the qualitative and quantitative distribution of covalently bound adducts to nucleic acids in high and low risk tissues in the hamster and rat upon i.g. and s.c. administration of DAN-3H; (4) determination of the qualitative and quantitative persistence of specific alkylated components of nucleic acids in high and low risk tissues in both species, as determined from the proposed chronic animal testing.

101. STUDIES ON N-NITROSO COMPOUNDS

Mirvish, S. S., University of Nebraska, Medical Center, School of Medicine, Dept. of Biochemistry, *42nd & Dewey Aves., Omaha, Nebraska,* 68105, United States of America

This program is planned to broaden our current knowledge of carcinogenesis by N-nitroso compounds in three main areas: (1) N-Nitroso compounds may be involved in the etiology of a significant fraction of human cancer. (2) N-Nitroso compounds are capable of inducing tumors in specific organs of experimental animals, which are difficult or impossible to induce by other carcinogens. (3) N-Nitroso compounds may act as carcinogens (at least in part) by alkylating cell macromolecules (e.g., DNA) in a fairly direct fashion, with or without prior enzymic activation.

102. ENVIRONMENTAL CARCINOGENESIS

Wynder, E. L., American Health Foundation Inc., *320 E. 43rd St., New York, New York,* 10017, United States of America

This program project grant has supported an integrated multidisciplinary program in environmental carcinogenesis relating retrospective case-control epidemiological studies and experimental research. The latter includes cell culture, animal model, and human subject investigations. Proposed studies by organizational division in the forthcoming period are: 1) Epidemiology: various cancer studies in the U.S. and overseas, emphasizing GI tract and breast cancer. 2) Nutrition: endocrine-related cancer studies via hormonal effects of high-fat diets in various human populations; also dietary studies on colon carcinogenesis utilizing fecal analyses. 3) Molecular Biology and Pharmacology: study of the mechanism of the high-fat effect on breast cancer development using animal models and cell cultures; also the mechanism by which alcohol may act as a cocarcinogen in oral cavity cancer. 4) Experimental Pathology: model systems for the study of the pathogenesis of tumor development. 5) Experimental Carcinogenesis: investigation of the carcinogenic action of hydrazines, nitrosamines, and methylated polynuclear hydrocarbons, in addition to bracken fern. 6) Occupational Health and Toxicology: assessment of cancer risk to welders as a model population.

103. MACHINE OILS AND NITROSAMINES - CARCINOGENESIS (NCI)

Neimeier, R., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Biomedical & Behavioral Science, *4676 Columbia Pkwy., Cincinnati, Ohio,* 45226, United States of America

The purpose of this study is to determine the carcinogenic potential of machine oils that are known to contain nitrosamines. Zingmark and Rappe (1976) reported the presence of diethanolnitrosamine in a grinding fluid. Fine (1976) confirmed these findings in a study where he randomly sampled 'off the shelf' synthetic cutting fluids and found a range of 400-1000 ppm diethanolnitrosamine. The presence of this nitrosamine is thought to be the result of adding amine emulsifiers (triethanolamine) and nitrite rust inhibitors to the oil formulation.

NIOSH estimates that approximately 780,000 workers are potentially exposed to these lubricating and cooling fluids during the processes of cutting and grinding metals. Reports of epitheliomas of the hands, arms, and scrotum in some of these workers have been noted by Decoufle (1976). In addition, colon and rectal cancer incidences are elevated in metal workers (Berg and Howell, 1975). The identity of the carcinogenic agent(s) in these occupational situations is not known.

Five unused synthetic machine oils and three laboratory formulations, containing known amounts of diethanolnitrosamine, as determined by chemical analysis, are being assessed for their carcinogenic potential by topical skin application on mice. Two positive control groups are included: 1) 3% diethanolnitrosamine in a nitrosamine-free synthetic cutting fluid, and 2) 0.2% benzo(a)pyrene. Triethanolamine in water is used to treat vehicle control group. In addition, a classical promotion experiment with croton oil is included. After 18 month treatment or at any time when a tumor is diagnosed grossly as a carcinoma and/or the animal is moribund, the animal is examined for pathological changes. Mean

latent periods, tumor types and numbers and relative carcinogenic potency (PMC) will be reported for each group and sex. This study is being carried out via the contractual route.

VIII. NITROSO COMPOUNDS IN THE ENVIRONMENT

A. ENVIRONMENTAL NITRITES AND NITRATES AND THEIR RELATION TO FORMATION OF NITROSO COMPOUNDS

104. INVESTIGATION OF FACTORS AFFECTING DEGRADATION OF BILE ACIDS AND FORMATION OF NITRITE-INDUCED MUTAGENS BY FECAL BACTERIA

Macdonald, I. A., Forrest, T. P., Stuttard, C., Rao, B. G., Clinical Research Centre, Dept. of Medicine, *5849 University Ave., Halifax, Nova Scotia*, B3H 4H7, Canada

OBJECTIVES: (1) To investigate the relationships between human cholesterol intake (with the fat:carbohydrate:protein ratio and calorie intake constant), nitrite induced mutagen formation in human feces and fecal bacterial enzymes (particularly 7 alpha-hydroxysteroid dehydrogenase). (2) To investigate the effect of altering the dietary carbohydrate:protein:fat ratio on nitrate-induced mutagen formation in human feces and fecal bacterial enzymes. (3) To obtain data useful in the identification and purification of fecal mutagens. (4) To investigate the possibility of nitrite inducible mutagens evolving from mixed fecal cultures and various culturing conditions (bile salts, reducing agents, pH and other media variables). (5) To investigate the possibility of nitrite inducible mutagens evolving from pure and binary bacterial cultures.

APPROACH: Feces are collected from healthy volunteers who are put on short-term diets (above). We will study the enzymes from fecal bacteria grown in vitro from a small specimen. Concurrently we will study the nitrite-induced mutagen production by the same sample. We will additionally introduce substances in vitro which can modulate the mutagenic response (bile salts, reducing agents, etc.).

105. CHEMICAL CARCINOGENS IN MAN

Archer, M. C., Bruce, W. R., Ontario Cancer Inst., *500 Sherbourne St., Toronto, Ontario*, M4X 1K9, Canada

The objective is to test the following hypothesis: 'Mutagens, including N-nitroso compounds, which are endogeneously produced in the lower gastrointestinal tract, are responsible for colon cancer and other tumors associated with Western diet.' We propose to test the hypothesis by examining the relationship of the three links: A) Between diet and formation of mutagens, B) between mutagens and cancer, and C) between diet changes that affect mutagen levels and the development of cancer.

Specifically, we plan: A) To determine dietary modifications that result in significant changes in the level of nitrate, nitrite and N-nitroso compounds in human feces and urine and to study the mechanism of nitrogen metabolism and formation and inhibition of nitroso compound production in feces cultured in vitro. B) To isolate and characterize mutagenic compounds that occur in man, to study their biological activity in animals, and to examine the relation between these compounds and human cancer. C) To determine whether dietary modifications that lead to reductions in the levels of mutagenic compounds in man, lead to a reduction in the development of preneoplastic lesions, genetic damage in target tissues and, ultimately, a reduction of cancer in the population.

106. EPIDEMIOLOGIC STUDY OF DIET AND STOMACH CANCER

Jain, M., Miller, A. B., Howe, G. R., Fodor, G., Choi, N. W., University of Toronto, Dept. of Preventive Medicine & Biostatistics, *92 College St., Toronto, Ontario*, M5S 1B2, Canada

OBJECTIVE: The objective of the present study is to investigate the hypothesis that dietary factors are related to stomach cancer and specifically that nitrosamines are formed in the stomach from the interaction of amines and nitrites of dietary origin. Factors known to increase or reduce the formation of nitrosamines will be investigated.

APPROACH: It is proposed to conduct a case/control study in three different provinces of Canada including approximately 400 newly diagnosed cases of stomach cancer and an equal number of population controls. Information will be collected by means of personal interviews using a personal and medical history questionnaire and a dietary history questionnaire. The dietary information collected will include the frequency and amount of consumption of various foods and also any changes from the present intake. In addition, the patterns or combinations of foods consumed for different meals will be assessed to seek the role of supposedly protective foods like vegetables and fruits.

107. ANALYSIS OF URINES FOR NITROSAMINES FROM PATIENTS WITH BILHARZIASIS

Hicks, R. M., Elsebai, I., Walters, C., El Nasser, A. B., University of London, Middlesex Hospital Medical School, School of Pathology, *Riding House St., London W1P 7LD, England, United Kingdom*

There is a high bladder cancer incidence in Egypt, in areas irrigated by canals from the Nile, and it is always linked with endemic bilharziasis. Bilharzial patients also frequently have chronic intractable bacterial infections of the urinary bladder. Many bacteria are capable of reducing nitrate to nitrite, and then nitrosating secondary and tertiary amines to nitrosamines. Secondary amines are normally in human urine. I have suggested, therefore, that nitrosamines could be a factor in the incidence of bladder cancer in Egypt, and if it is so, the bilharziasis is a co-factor, rather than a direct carcinogen. A pilot study has been undertaken with the collaboration of IARC, in which urines from bilharzia infested patients in Cairo and surrounding districts were analyzed by gas chromatography and mass spectrometry. Many of these patients had significantly higher levels of several nitrosamine species in their urines than did uninfested Egyptian and European individuals. For comparison, urines of European patients with chronic bacterial infections of the urinary tract were analyzed in the same way. This investigation will now be extended to other areas where bilharziasis is endemic.

108. ENDOGENOUS NITRITE CARCINOGENESIS IN MAN

Tannenbaum, S. R., Fox, J. G., Gomez, R. F., Naum, K. M., Newberne, P. M., Rogers, A. E., Skipper, P. L., Thilly, W. G., Ulmer, K. M., Wishnok, J. S., Wurtman, R. J., Young, V. R., Massachusetts Inst. of Technology, School of Science, Dept. of Nutrition & Food Science, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

It is proposed to investigate the role that nitrite and N-nitroso compounds may play in the etiology of human cancer. The focus of the program is on determining the endogenous synthesis and flux of precursors of nitrite and nitrosatable N-compounds in the gastrointestinal tract of man, and the influence of diet on formation, stability and reactivity of nitrite. A major goal is to develop an analytical approach to evaluation of the specific risk for an individual from endogenous nitrosation reactions. A second major goal is to develop information which will lead to dietary prevention of endogenous nitrosation. These studies will be conducted in man and experimental animals, and will integrate the skills of a wide variety of scientific disciplines.

109. DIET AND INTESTINAL FORMATION OF NITRITE IN MAN

Tannenbaum, S. R., Young, V. R., Gomez, R., Massachusetts Inst. of Technology, School of Science, Dept. of Nutrition & Food Science, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

Studies of nitrate balance in man and analyses of fecal and ileostomy samples indicate that nitrite and nitrate are formed de novo in the intestine, possibly by heterotrophic nitrification. These findings significantly alter our previous conceptions of man's exposure to nitrite and suggest that nitrite may play a role in the etiology of intestinal cancer via the formation of carcinogenic N-nitroso compounds. In the proposed research, we will conduct experiments to determine the effect of diet on formation of nitrite and N-nitroso compounds in man. These experiments will be performed on normal, healthy individuals, and on patients with ileostomies. We will explore the microbial formation of nitrite from ammonia and determine whether dietary

variables including fat, protein, fiber, ascorbic acid, and alpha-tocopherol influence the formation of nitrite and its reactivity.

110. STUDY OF ENVIRONMENTAL OCCURRENCE OF N-NITROSO COMPOUNDS

Tannenbaum, S. R., Massachusetts Inst. of Technology, School of Science, Dept. of Nutrition & Food Science, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

OBJECTIVES: Determine whether a direct relation can be established between formation of nitrite in the human stomach and gastric cancer. The types of studies to be conducted are as follows: characterization of the gastric contents of individuals from a high-risk gastric cancer area in comparison with a low-risk area; identification of factors controlling rate of nitrosation in human gastric juice; and characterization of mutagenic N-nitroso compounds formed from predominant Colombian foods.

111. FORMATION OF N-NITROSO COMPOUNDS AND BIOCHEMICAL STUDIES ON THE ESOPHAGUS

Mirvish, S. S., University of Nebraska, Medical Center, School of Medicine, Dept. of Biochemistry, *42nd & Dewey Aves., Omaha, Nebraska*, 68105, United States of America

N-Nitroso compounds occurring in the environment or formed in vivo might be responsible for some human cancers, e.g., gastric cancer. Ascorbate and other antioxidants occurring naturally or added to food and drugs may inhibit the formation of these compounds. Hence we propose to study: 1) Methylnitrosourea formation in the stomach contents of rats fed methylurea and nitrite, and the effect of antioxidants on this formation; 2) alkylation of rat stomach nucleic acids, after feeding of methylurea and nitrite; 3) nitrosamide formation in organic solvents and organic solvent:water mixtures, and the effect of antioxidants on this formation; 4) the occurrence of urea compounds in foods and feces after nitrosation-denitrosation; 5) induction of lung adenomas by primary aromatic amines and nitrite; 6) effect of ascorbate on tumor induction in rats by morpholine and nitrite.

112. IN VIVO NITROSAMINES AND CANCER IN RENAL FAILURE

Simenhoff, M. L., Thomas Jefferson University, Jefferson Medical College, Dept. of Medicine, *1025 Walnut St., Philadelphia, Pennsylvania*, 19107, United States of America

N-nitroso compounds (nitrosamines) are potent mutagens and carcinogens in experimental animals. Although suggestive, no role has been determined in man. A chance observation while investigating amine generation in chronic renal failure pointed to a human model in uremic patients who have been reported to have an increased incidence of malignancies. This model describes the coexistence of 3 conditions for nitrosamine formation: 1) intestinal nitrates (nitrites), endogenous and exogenous (in food and water, including dialysate), 2) small intestinal secondary amines, especially dimethylamine (DMA), and 3) appropriate bacterial species in same location permitting reduction of nitrate and possibly nitrosation itself, at physiologic pH and temperatures. The proposed research will identify the extent to which these three conditions exist and determine whether nitrosamine (specifically dimethylnitrosamine) is formed in vivo. If nitrosamine formation is significant a small series of patients given oral chemical inhibitors will be compared for successful inhibition of nitrosamine production. The emphasis here is on human in vivo measurement rather than on environmental pathogenesis (N-nitroso compounds in food, sewerage treatment wastes, soil, water, urban air, cigarette smoke) except as it is relevant to endogenous formation or its augmentation. Methods to be used have all been developed, some quite recently, with much of the local equipment and expertise to carry out the program. N-nitroso-compounds will be measured with gas chromatography/thermal energy analyzer, aliphatic amines by gas chromatgraphy and nitrates and nitrates by a modified Greiss method.

Additionally, the incidence of malignancy will be determined on a national basis in patients with end stage uremia through existing national registers and a direct questionnaire to dialysis units. Selected patients from this compilation will then be studied further. This information is particularly important in view of the federal commitment to chronic dialysis which is offered to the population at large, and which has extended their period at risk from cancer.

B. NITROSO COMPOUNDS IN FOODS

113. CANCER RISK IN VEGETARIANS

Frentzel Beyme, R. R., Preussmann, R., Deutsches Krebsforschungszentrum, Institut fuer Dokumentation, Information und Statistik, *Im Neuenheimer Feld 280, 6900 Heidelberg, Federal Republic of Germany*

Animal experiments have shown many nitrosamines to be carcinogenic. Nitrosamine precursors in human food and vegetables have been investigated and high concentrations in certain vegetables suggest the possibility of a higher risk of cancer in persons consuming particular types of greens. Analytical studies together with a morbidity and mortality survey of a cohort of vegetarians are being undertaken to investigate a postulated relationship between cancer incidence and dietary habits. Control groups will be comprised of persons who have also willingly chosen a so-called 'reformed' life style (i.e., preference of a natural diet, but not exclusively vegetarian) as well as industrial employee populations considered to live on a conventional diet and being otherwise comparable (education, age, sociological background).

114. STUDY OF FOOD-BORNE ENVIRONMENTAL TOXICANTS

Wogan, G. N., Tannenbaum, S. R., Karel, M., Thilly, W. G., Sinskey, A. J., Marletta, M., Brunengraber, H., Newberne, P. M., Rogers, A. E., Wishnok, J. S., Massachusetts Inst. of Technology, School of Science, Dept. of Nutrition & Food Science, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

This is a broad program of research encompassing a range of problem areas and involving a diversity of experimental approaches. It is therefore important that this progress report be organized according to the conceptual framework of the program, which is described in the following outline: Program Area I: Segments Utilizing Chemical Models: A. Nitrosamines and Nitrosation Products: B. Reactions of Peroxidizing Lipids with Proteins and Amino Acids; Program Area II: Segments Utilizing Cell Culture Models: A. Human and Other Cell Cultures; B. Bacterial Systems for Investigating DNA Damage and Repair. Program Area III: Segments Utilizing Animal Models: A. Biochemical Toxicology of Perfused Organs and Isolated Hepatocytes; B. Interactions Among Environmental Agents. Program Area IV: Tissue Responses and Subcellular Interactions: A. Carcinogen Effects on Nuclear Function and Other Biochemical Processes; B. Carcinogen-Induced Modifications of Cellular Macromolecules.

115. N-NITROSAMINES IN FOODS

Scanlan, R. A., Libbey, L. M., Sinnhuber, R. O., Oregon State Higher Education System, Oregon State University, School of Agriculture, Dept. of Food Science & Technology, *35th & Jefferson Sts., Corvallis, Oregon*, 97331, United States of America

The overall objective of our research is to investigate the occurrence and formation of N-nitrosamines in food systems and to evaluate the hazard of the compounds formed. The specific research goals for the upcoming year include use of a high performance liquid chromatographic-thermal energy analyzer to develop methods for analysis of nonvolatile N-nitroso compounds in foods. In addition, current methodology will be used to screen selected foods for volatile nitrosamines. Projects will also be designed to elucidate the factors responsible for the formation of nitrosopyrrolidine and dimethylnitrosamine in certain foods.

BIBLIOGRAPHIC REFERENCES: Hotchkiss, J.H., Barbour, J.F., Libbey, L.M., and Scanlan, R.A. 1978. Nitramines as Thermal Energy Analyzer Positive Nonnitroso Compounds Found in Certain Herbicides. Agricultural and Food Chemistry 26:4:884. Grieco, M.P., Hendricks, J.D., Scanlan, R.A., Sinnhuber, R.O., and Pierce, D.A. 1978. Carcinogenicity and Acute Toxicity of Dimethyl-nitrosamine in Rainbow Trout (Salmo gairdneri). J. Natl. Cancer Inst. 60:5:1127-1131.

C. OTHER STUDIES OF NITROSO OR NITROSABLE COMPOUNDS IN THE ENVIRONMENT (INCLUDING DRUGS)

116. N-NITROSO COMPOUNDS AND PRECURSORS IN THE ENVIRONMENT

Walters, C. L., British Food Manufacturing Industries Research Assn., *Randalls Rd., Leatherhead, England, United Kingdom*

The work called for by Items 1-3 shall be performed in accordance with the Contractor's Technical Proposal dated January 16, 1975, entitled 'Method of Determination of Non-Volatile N-nitroso Compounds in Biological Matrices'. The Contractor's Technical Proposal referenced above is incorporated into and made a part of this contract as though fully set forth herein; except, however, in the event of a conflict between said Technical proposal and any other terms and conditions of this Supplemental Agreement then all other terms and conditions of this Supplemental Agreement shall take precedence over the Contractor's Technical Proposal.

117. CARCINOGENIC NITROSAMINES FROM PRIMARY AMINES

Tannenbaum, S. R., Wishnok, J. S., Skipper, P. L., Massachusetts Inst. of Technology, School of Science, Dept. of Nutrition & Food Science, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

Nitrosamines are well-known chemical carcinogens, some of which have been implicated in the etiology of human cancer. The objective of this research is to determine whether nitrosation of primary amines might contribute to the exposure of man to these compounds, particularly via in vivo nitrosation in the gastrointestinal tract. We will conduct experiments to determine whether nitrosation of alkyl and aryl primary amines can lead to alkylation of cellular nucleophiles, cause mutation in bacteria, or condense with other N-compounds to yield novel N-nitroso compounds.

118. MEDICAL, MORTALITY AND INDUSTRIAL HYGIENE STUDY OF WORKERS

Fajen, J., Egan, B., Smith, B., Fine, D., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance, Hazard Evaluations & Field Studies, *4676 Columbia Pkwy., Cincinnati, Ohio*, 45226, United States of America

Dimethylnitrosamine is labelled as a carcinogen by the Occupational Safety and Health Administration and therefore in the few areas where it is used or manufactured, appropriate precautions are taken. It has only recently been discovered through the development of new analytical techniques that dimethylnitrosamines and other nitroso compounds may form in the ambient atmosphere or even in vivo if sufficient concentrations of nitroso compound precursors (secondary amines, amides, etc.) and nitrates, nitrites, or other nitrogenous compounds exist under appropriate conditions.

Twenty-one industrial plants have been investigated for any possible nitrosamine contamination. The industries visited thus far have been the automobile, azo dye, small machine shops, fish and fish meal, rubber and leather. To date N-nitrosodiethanolamine, N-nitrosodimethylamine, N-nitrosomorpholine, and N-nitrosodiphenylamines have been found in the air of several plants. N-nitrosodimethylamine, a regulated carcinogen, was found as high as 50 micrograms/cubic meter in the leather industry. Efforts are being made to determine the nitrosating agent, the extent of human exposure, and methods of controlling the exposure to N-nitrosodimethylamine.

Detailed industrial hygiene and biological sampling will be conducted in the rubber and leather industries as well as newly identified industries to determine the level and extent of nitrosamine exposure and to identify conditions necessary for spontaneous formation of these compounds.

An ophthalmologic survey was undertaken at the E.I. duPont de Nemours and Company, Belle, West Virginia plant, where an excess of eye tumors has been noted and where nitrosamines have been found in the ambient environment. Data analysis will proceed in 1979, and follow-up studies of participants in that survey will be conducted.

IX. CHEMISTRY, SYNTHESIS, AND REACTIONS OF NITROSO COMPOUNDS

119. FREE RADICAL REACTIONS IN CHEMICAL CARCINOGENESIS

Stock, L. M., University of Chicago, School of Physical Sciences, Dept. of Chemistry, *5734 S. Ellis Ave., c/o Hinds Geophysics Lab., Chicago, Illinois*, 60637, United States of America

This research concerns the reactions of the N-alkyl-N-nitrosoureas with the constituents of the nucleic acids. In the next year additional attention will be given to the reactions of diazonium compounds with adenine and guanine and their derivatives. The reaction pathways will be determined.

BIBLIOGRAPHIC REFERENCES: J.K. Snyder and L.M. Stock, Conformational Preferences in Alkylnitrosoureas, J. Org. Chem., 44, 0000 (1979), in press.

120. CANCER AND ANTICANCER ACTIVITY OF NITROSO COMPOUNDS

White, E. H., Johns Hopkins University, School of Arts & Sciences, Dept. of Chemistry, *34th & Charles Sts., Baltimore, Maryland*, 21218, United States of America

We plan to synthesize and have tested a selection of N-nitrosoamides, N-nitrosocarbamates, and N-nitrosoureas and several of the corresponding N-nitro compounds. These compounds are designed to form carbonium ions in solution, ions which are probably the active species in the carcinogenic and anticancer reactivities of the chloroethylnitrosoureas; we plan to examine new types of precursors which will deliver new types of carbonium ions. Diazotates, nitrososulfamates, and nitrosoamides of amino acids will be examined with the objective of separating the anti-cancer and mutagenic activities. Attention will be paid to the mechanism of carbonium ion formation by these compounds. The compounds will be tested for carcinogenicity, mutagenicity, and anticancer activity.

BIBLIOGRAPHIC REFERENCES: Arylmonoalkyl and Cyclic Triazenes: Direct Acting Mutagens, H.F. Thomas, D.L. Brown, P.E. Hartman, and Emil H. White, Mutation Research, 60, 25-32 (1979). A Comparison of Mutagenicity, Antitumor Activity, and Chemical Properties of Selected Nitrosureas and Nitrosoamides, Robert B. Bundrett, Michael Colvin, Emil H. White, James McKee, Philip E. Hartman and Diane L. Brown, Cancer Research, 39, 1328-1333 (1979).

121. CHEMISTRY OF N-NITROSO COMPOUNDS

Keefer, L. K., Roller, P. P., Angeles, R. M., Fanning, J., Slavin, B., Hillery, P., Michejda, C., Sansone, E., Rice, J., Kleihues, P., Emmett, G., Doeryer, G., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Analytical Chemistry Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Data concerning the chemical and physical properties of the carcinogenic N-nitroso compounds will be collected. Specific emphasis will be placed on studies of: a) catalysis of N-nitrosation reactions of environmental interest by electrophilic species, including transition metal complexes; b) development and evaluation of nitrosamine degradation methods potentially applicable to laboratory problems in carcinogen decontamination and disposal; c) preparation of novel nitrosamines and their derivatives for chemical and biological studies. Possible implications of this work with respect to the overall goal of human cancer prevention will be considered.

BIBLIOGRAPHIC REFERENCES: Angeles, R.M., Keefer, L.K., Roller, P.P., and Uhm, S.J.: Chemical models for possible nitrosamine artifact formation in environmental analysis. In Walker, E.A., Castegnaro, M., Griciute, L., Lyle, R.E. (Eds.): Environmental Aspects of N-Nitroso Compounds. IARC Scientific Publication No. 19. Lyon, 1978, pp. 109-115. Keefer, L.K.: Alpha-amino nitrite esters and their analogs - Possible reactive intermediates in nitrosamine formation? In Gould, R.F. (Ed.): ACS Symposium Series, Bk. 101, Chapter 6, 1979, in press.

122. NITROSAMINE FRAGMENTATION AND NITROSAMINE CARCINOGENESIS

Loeppky, R. N., McKinley, W., University of Missouri, Columbia Campus, School of Arts & Sciences, Dept. of Chemistry, *130 Jesse Hall, Columbia, Missouri*, 65201, United States of America

Numerous studies have reported beta-hydroxynitrosamines, as well as their oxo derivatives to be metabolites of nitrosamines in vivo and in vitro. Beta-Hydroxynitrosamines can be produced in the environment from a wide variety of ubiquitous beta-hydroxyamines. It has been proposed elsewhere that beta-ketonitrosamines undergo biochemical fragmentation to simpler nitrosamines which eventually alkylate nucleic acids. Previous work in these laboratories has led to the discovery of a facile chemical cleavage of beta-hydroxy- and beta-acetoxynitrosamines. Here we propose to elucidate the chemical fragmentation reactions of beta-hydroxy-, beta-keto-, and beta-acetoxynitrosamines and delta-ketonitrosamines (possible products of environmental tertiary amine nitrosation) by examining the response of the rate and extent of cleavage to structural features and the nature of catalysts in order to perceive the role that such transformations may play in the environmental production and destruction of nitrosamines in relation to their carcinogenicity and as a model for a biochemical study. A search for the biochemical counterparts to the foregoing transformations as well as a determination of the respective biochemical roles of beta-hydroxy- vs. beta-ketonitrosamines is also proposed in order to further elucidate the mechanisms of nitrosamine carcinogenesis. The examination of the possible addition of nucleophiles to enenitrosamines to produce fragmentable substances is also a part of this proposal as is the exploration of the fragmentation of oxidation products of beta-hydroxynitrosamines which could produce nitrogen containing metabolites. Further understanding of the relationship of these processes to nitrosamine carcinogenesis will be sought by utilization of the Ames Mutagen Assay to screen compounds.

123. STRUCTURAL REQUIREMENTS FOR NITROSAMINE CARCINOGENICITY

Jacobson, M. K., Lyle, R. E., North Texas State University, School of Arts & Sciences, Dept. of Chemistry, *N. Texas Station, Box 13707, Denton, Texas*, 76203, United States of America

The relative carcinogenicity of a number of conformationally biased N-nitrosamines appears to be related to the availability of an hydrogen alpha to the nitrosamine function and in a plane perpendicular to that containing the nitrosamine and its substituents. This proposal is designed to test this apparent correlation by structure/function studies of a number of novel N-nitrosamines. A detailed study of an apparent exception to this correlation, N,N-dibenzylnitrosamine, is proposed. The N-nitrosamines will be examined for their mutagenic potential in the Ames assay directly and in the presence of a metabolic activation system. Carcinogenicity of selected compounds will be examined in whole animal studies. The significance of the interaction of N-nitrosamines with polyhydroxy compounds such as ascorbic acid and sucrose will also be studied.

X. ANALYTICAL METHODS FOR NITROSO COMPOUNDS

124. STEREOCHEMICAL STUDY OF TOXIC NITROGENOUS COMPOUNDS IN FOODS

Gaffield, W., U.S. Dept. of Agriculture, Federal Research Office, Western Regional Research Center, *800 Buchanan St., Albany, California*, 94710, United States of America

OBJECTIVE: Develop chemical ionization mass spectrometric techniques for the analysis and confirmation of structure of nitrosamines and related toxic compounds.

APPROACH: Model nitrosamino acids will be synthesized. Stereochemical properties of N-nitrosamines will be studied. Chemical ionization mass spectrometry will be used for the analysis and confirmation of structures of N-nitrosamines present in natural systems. Information obtained will be used to improve the analytical methodology needed in studies of a wide variety of toxic nitrogenous compounds found or formed in foods.

PROGRESS: N-Nitrosodibutylamine (DBN) and fully deuterated N-nitrosodimethylamine (DMN-d(6)) have been successfully tritiated using the Wilzbach procedure, i.e., exposure to tritium gas. Specific activities, after purification and transfer to aqueous solution for biochemical studies, were 180 and 14 millicuries/millimole for DBN and DMN-d(6), respectively. 3H-N-Nitrosodibutylamine (3H-DBN) was rather sensitive to autoradiolysis, although the unlabelled derivative proved unusually resistant to base-catalyzed exchange with deuterium oxide. No decrease in deuterium content was observed during the preparation of 3H-N-nitrosodimethylamine-d(6) (3H-DMN-d(6)), demonstrating that tracer quantities of tritium could be incorporated without significantly lowering the deuterium isotopic purity. These results demonstrate that the Wilzbach procedure may serve as a useful alternative to base catalyzed exchange for the synthesis of tritiated nitrosamines. Nuclear magnetic resonance (NMR) data were reported for several hexafluoroacetone (HFA) adducts of nitrosamino alcohols and amines. Notable differences in the spectra of HFA adducts of positional isomers are evident with N-nitroso-3-piperidinol and N-nitroso-4-piperidinol and in the spectra of HFA adducts of stereoisomers N-nitroso-trans-4-hydroxyproline and N-nitroso-cis-4-hydroxyproline. By the use of Fourier transform NMR, about 0.3 ng of the HFA adduct of mono-N-nitrosopiperazine was detected in 0.4 ml of solvent.

125. ELECTROANALYTICAL STUDIES OF N-NITROSAMINES

Harrington, G. W., Magee, P. N., Swern, D., Temple University, School of Liberal Arts, Dept. of Chemistry, *Broad St. & Montgomery Ave., Philadelphia, Pennsylvania*, 19122, United States of America

The proposed research is directed towards the further development of precise, accurate and sensitive electroanalytical methods for volatile and non-volatile N-nitrosamines and the nitrosamine precursors nitrate and nitrite. The methods must be capable of analyzing a wide variety of complex matrices, including various body fluids. They must also be useful for in situ studies and be applicable to the analysis of small samples.

The analytical methods will be applied to problems of significant chemical and biological interest such as studies designed to investigate the role of ascorbic acid and alpha-tocopherol in the formation and possible destruction of N-nitrosamines. Rates of metabolism and the effect of metabolic inhibitors will be studied using single animal techniques plus the electroanalytical methods.

A simple sensitive method for total nitrosamine analysis will be developed that is capable of treating small samples. The method will be used to study various possible routes of endogenous formation of N-nitrosamine, including comparison of nitrosamine content of portal blood with that of ciruclating blood. The new electrochemical detector for high performance liquid chromatography (HPLC) will be evaluated and compared with the thermal energy analyzer detector.

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D. If you are submitting a clinical protocol, please send a copy of the complete protocol. The NCI has an established format which will be used to develop a standard protocol summary. A copy of the prepared summary will be sent to you for review prior to its entry into the data base.

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